REMARKS

Applicants respectfully request reconsideration of the present application in view of the following remarks.

I. Status of the Claims

Claims 1-22 and 25-54 are currently pending in the application, with claims 1, 30 and 35 being the independent claims.

Claims 23-24 were previously cancelled without prejudice or disclaimer to the subject matter therein.

Claims 1, 3-4, 10-12, 16, 19, 21, 30, 32-34 and 53-54 are amended to specify that the rate-controlling polymer is a high molecular weight rate-controlling polymer. Support for the amendment to claims 1, 3-4, 10-12, 16, 19, 21, 30, 32-34 and 53-54 may be found, *inter alia*, in Examples 1-2, 5 and 7 of the specification. These amendments do not introduce any new matter into the application and their entry is respectfully requested.

II. The Rejection Under 35 U.S.C. § 103(a)

The Advisory Action, at page 2, maintains the rejection of claims 1-22 and 25-53 under 35 U.S.C. § 103(a) as being allegedly unpatentable over U.S. Patent No. 5,573,783 to Desieno *et al.* ("Desieno") and U.S. Patent No. 5,145,684 to Liversidge *et al.* ("Liversidge"), in view of U.S. Patent No. 5,811,388 to Friend *et al.* ("Friend"). Specifically, the Advisory Action alleges that Desieno teaches the use of the same rate-controlling polymers as the invention and shifts the burden to Applicants to show that the nanoparticulate system disclosed by Desieno does not exhibit controlled-release properties. Applicants respectfully traverse this ground of rejection.

The Advisory Action also rejects Applicants' arguments regarding the differences in molecular weight between the polymers listed in Desieno and the polymers of the claimed invention, noting that the high molecular weight feature is not recited in the claims.

Applicants wish to thank the Examiner for suggesting to include the molecular weight limitation in the claims. Thus, in order to advance prosecution, and without acquiescing to the propriety of the rejection, Applicants have amended the claims to specify that the rate-controlling polymers are high molecular weight polymers. As stated above, support for the amendment to the claims may be found, *inter alia*, in the examples of the disclosure.

Example 2 in the specification, for example, provides a controlled-release nanoparticulate naproxen formulation using a mixture of Klucel and PVP as controlled-release polymer. The PVP used in the Example is Plasdone K-90. Plasdone K-90 has an average molecular weight of 1,300,000 (*see* Physical and Chemical Properties of Plasdone Povidone, attached as Exhibit A). In addition, all other polymers used in the examples in the application, such as Klucel and Methocel, have also high molecular weight. Thus, Klucel has an average molecular weight between 80, 000 and 1,150,000 (see Klucel, attached as Exhibit B), Methocel 4M has an average molecular weight of 95,000 (*see* Baumgartner *et al. Pharmac. Res. 19(8)*: 1084-90 (2002), attached as Exhibit C), and Methocel E4M has an average molecular weight of 86,000 (*see* Shah and Donovan *AAPS PharmSciTech 8 (2)*, Article 32, attached as Exhibit D).

In contrast to Applicants' claimed invention, Desieno discloses PVP/PEG polymers having an average molecular weight of 7,000 at the most, and describes polyethylene glycols having average molecular weights in the range of 300 to 8,000, with polyethylene glycol 3350 being the preferred PEG of the invention. *See* col. 5, lines 22-25. Thus, the Povidone (k15/17) used in Example 3 of Desieno, for example, has an average molecular weight of 10,000 (see Exhibit A).

As stated in the Reply filed on February 12, 2007, it is well known in the art that the presence of polyethylene glycols having low average molecular weight, such as an average molecular weight in the range of 300 to 8,000, in drug formulations causes a fast drug dissolution rate, because the polymer is in a fluid state and maintains the fluidity of the drug composition when water is imbibed into the drug matrix. High molecular weight polyethylene glycols, high molecular weight PVP, high molecular weight plant exudates or

enteric polymers, such as those used in the present application, on the other hand, after imbibing water tend to be viscous and less fluid, thus slowing the drug dissolution rate and providing drug controlled release.

The claimed nanoparticulate compositions are specifically designed to prevent rapid dissolution of the drug and *provide controlled drug release by including a rate-controlling polymer in the nanoparticulate formulations*. Accordingly, at least because of the reasons stated above, Desieno fails to teach or suggest a *rate-controlling polymer* integrated in a rate-controlling matrix with the drug composition, or coating the nanoparticulate drug composition, as recited in the claimed invention.

As stated in the Reply filed on February 12, 2007, the additional references cited in the rejection, Liversidge and Friend, do not remedy the deficiencies of Desieno.

Liversidge teaches against controlled release nanoparticulate compositions, because Liversidge teaches that the aim of controlling the size and the size range of drug particles is *to increase the rate of dissolution, and thus to increase the release rate of the nanoparticulate drug*. *See* col. 1, lines 28-33. Accordingly, Liversidge, like Desieno, discloses immediate release drug nanoparticulate compositions, and fails to teach or suggest controlled release nanoparticulate compositions.

Friend specifically teaches that the compositions and methods of the invention are of a delayed release nature, as compared to sustained or extended release, and states that "*a delayed release composition allows for the release of most of the active ingredient in the lower GI and... is different than a sustained release composition that releases the active on a regular (i.e. constant) basis throughout the GI.* *See* col. 4, line 66 to col. 5, line 6. Thus, Friend, like Desieno and Liversidge, fails to teach or suggest controlled release nanoparticulate compositions.

Moreover, there is no reason to combine the references to attempt to arrive at the claimed invention, since, as stated above, Liversidge teaches against controlled release

nanoparticulate compositions, and Friend teaches compositions for delayed release, **not** for sustained or extended release

In essence, the cited prior art fails to disclose or suggest the unexpected pharmacokinetic properties of the nanoparticulate compositions of the present invention.

For at least these reasons, the rejection of claims 1- 22 and 25-54 under 35 U.S.C. § 103(a) is improper. Reconsideration and withdrawal of this ground of rejection are therefore respectfully requested.

CONCLUSION

All of the stated grounds of rejections have been properly traversed or rendered moot. Therefore, the present application is now in condition for allowance. Favorable reconsideration of the application is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 I.E. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check or credit card payment form being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicants hereby petition for such extension under 37 I.E. § 1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date July 6, 2007

By Michele M Simkin

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EXHIBIT A

Physical & Chemical Properties

Chemical Description

Chemical Description:	Polymer of 1-vinyl-2-pyrrolidone, Pharmaceutical grade
CAS Registry Number:	9003-39-8
CAS Registry Name:	1-ethenyl-2-pyrrolidinone homopolymer
Chemical Formula:	$(C_6H_9NO)_n$
Synonyms:	Polyvinyl pyrrolidone (PVP), Povidone, Polyvidonum, Poly (N-vinyl-2-pyrrolidinone), Soluble PVP

K-Value & Molecular Weight

Plasdone polymers are manufactured in a variety of molecular weights depending on the conditions of polymerization. Like many free-radical polymerization processes, the manufacturing process does not produce polymer of a single chain length or molecular weight. Instead, various chain length polymers exist. Therefore, molecular weight is reported as average molecular weight.

There are several methods used for determining average molecular weight and the molecular weight will vary based on the method used. The weight average molecular weight of the Plasdone products as measured by light scattering is reported in Table 1.

Because it is difficult to determine the molecular weight of the polymer directly, the K-value, has been adopted to classify the various molecular weights of Plasdone polymers. The K-value, a function of the average degree of polymerization and the intrinsic viscosity of the polymer, is calculated from the kinematic viscosity of a 1% w/v aqueous solution. The K-values for the various Plasdone products are also shown in Table 1.

Table 1. Plasdone polymers are available in a range of molecular weights and K-values.

	K-Value	Weight Average Molecular Weight (Mw)
Plasdone® K-12	10.2-13.8	4,000
Plasdone® C-15	15.5-17.5	10,000
Plasdone® K-17	16-17.5	10,000
Plasdone® K-25	24-26	34,000
Plasdone® C-30	29-32	58,000
Plasonde® K-29/32	29-32	58,000
Plasdone® K-90	85-95	1,300,000
Plasdone® K-90D	85-95	1,300,000

Particle Morphology

All of the Plasdone products, with the exception of Plasdone K-90 and K-90D polymers, are supplied as spray-dried, free-flowing white powders. Under scanning electron microscope (SEM), Plasdone particles appear spherical, thus they exhibit excellent flow properties and are well suited for powder blending. As an example, a SEM for Plasdone K-29/32 is shown in Figure 2.

Plasdone K-90 polymer is a fine, free-flowing, flaky powder (Figure 3). Plasdone K-90D polymer, although chemically identical to Plasdone K-90 polymer, is a densified form with more rounded particles (Figure 3) for easier and faster dispersion.

Figure 2. Plasdone K-29/32 polymer is a free flowing powder with spherical particles.

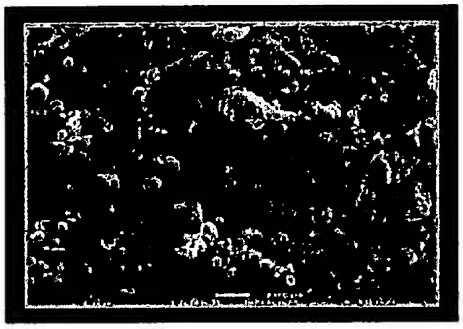
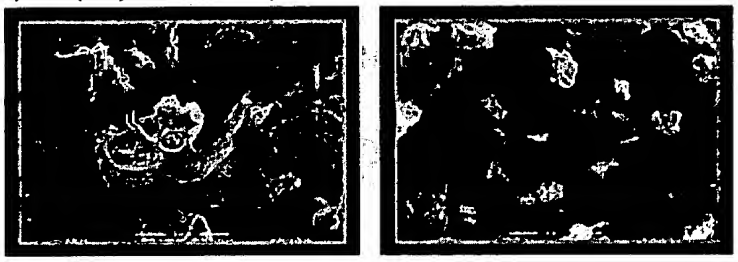


Figure 3. Plasdone K-90D polymer particles (right) are more rounded than Plasdone K-90 polymer (left) for faster dispersion.



Glass Transition Temperature (Tg)

The glass transition temperature of Plasdone polymers is dependent on the molecular weight of the polymer (Table 2). The lower molecular weight polymers will have lower Tg than high molecular weight polymers.

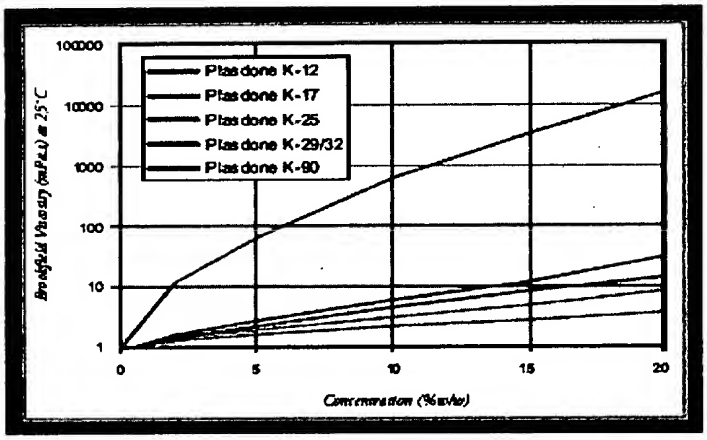
Table 2. The glass transition temperature of Plasdone polymers is dependent on the polymer's molecular weight.

	Tg (°C)
Plasdone® K-12	120
Plasdone® C-15	126
Plasdone® K-25	160
Plasonde® K-29/32	164
Plasdone® K-90	174

Solubility & Viscosity

Plasdone polymers are completely soluble in water at room temperature. The maximum concentration of polymer soluble in water is limited only by the viscosity at a given concentration. The viscosity of an aqueous solution depends on the molecular weight of the polymer, with the lower molecular weight products having the least effect on aqueous viscosity relative to the higher molecular weight materials (Figure 4).

Figure 4. The higher molecular weight Plasdone K-90 polymer provides the highest aqueous solution viscosity.



In addition to water, the hydrophobic/hydrophilic balance of Plasdone polymers makes them soluble in a wide variety of organic solvents at room temperature (Table 3). The viscosity of a solution is related to the viscosity of the solvent and will vary based on the solvent selected, the molecular weight of the polymer and the concentration.

Table 3. Plasdone polymers are soluble in a wide range of organic materials.

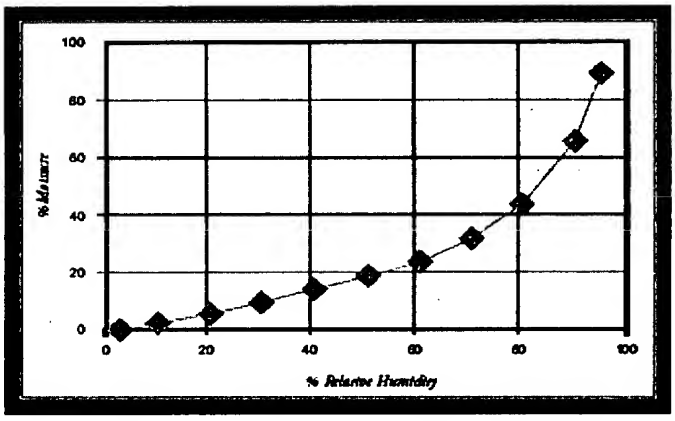
Soluble ***Plasdone polymers are soluble at greater than 10% w/w at room temperature***	
Acids	Acetic Acid Formic Acid Propionic Acid
Alcohols	Butanol Ethanol Methanol Propanol
Amines	Ethylene Diamine Triethanolamine (TEA)
Chlorinated Hydrocarbons	Chloroform Ethylene Dichloride Methylene Dichloride
Esters	Ethyl Lactate
Glycerol	
Glycols	Diethylene Glycol Polyethylene Glycol (PEG) 400 Propylene Glycol
Ketones	Methylcyclohexanone
Lactams	
Nitroparaffins	
Poorly Soluble or Insoluble ***Plasdone polymers are soluble at less than 10% w/w at room temperature***	
Chlorinated Hydrocarbons	Carbon Tetrachloride Chlorobenzene Tetrachloromethane
Esters	Ethyl Acetate Sec-butyl Acetate
Ethers	Diethyl Ether
Hydrocarbons	Light Petroleum Pentane Toluene Xylene
Ketones	Acetone 2-Butanone Cylcohexanone

Other	Mineral Oil

Hygroscopicity

Plasdone polymers absorb water independent of the molecular weight of the polymer. However, the level absorbed will depend on the relative atmospheric humidity (Figure 5). The equilibrium water content is equal to approximately one-third the relative humidity.

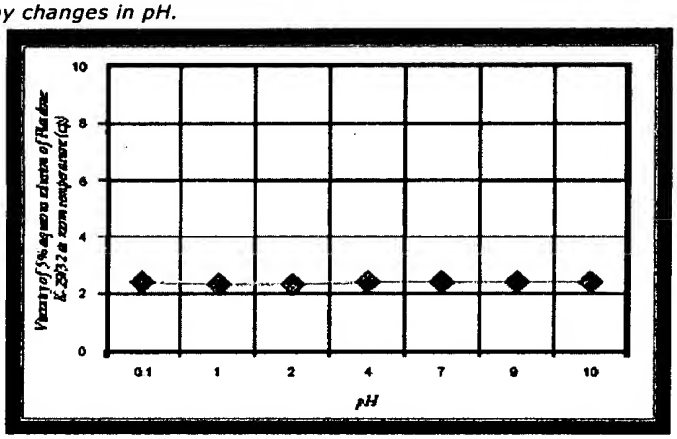
Figure 5. Plasdone K-29/32 polymer will absorb water relative to the relative humidity.



Compatibility

Plasdone polymers are compatible with a wide range of materials as demonstrated by their tolerance for pH changes and salts. The insensitivity to pH is demonstrated by the aqueous viscosity of a 5% solution of Plasdone K-29/32 polymer at various pH levels (Figure 6).

Figure 6. The viscosity of Plasdone K-29/32 polymer in aqueous solution is not affected by changes in pH.



Furthermore, the appearance of a 1% aqueous solution of Plasdone K-29/32 polymer is unaffected by the addition of 10 grams of the following salts at 25°C:

- Aluminum sulfate ($Al_2(SO_4)_3 \bullet 18H_2O$)
- Ammonium chloride (NH_4Cl)
- Calcium chloride ($CaCl_2$)
- Sodium chloride (NaCl)
- Sodium phosphate (primary) ($NaH_2PO_4 \bullet H_2O$)
- Zinc sulfate ($Zn(SO_4) \bullet 7H_2O$)

This would suggest that Plasdone polymers will not interact with ionic drug actives

or their salts.

Plasdone polymers are compatible in solution and in the solid state with a wide variety of materials. To determine compatibility, Plasdone K-29/32 and the test material were dissolved separately in a common solvent. After mixing the two solutions, the appearance was observed. The solution was then cast onto a glass plate and the resulting film examined after evaporation of the solvent. The polymer was compared to the test compound in ratios of 1:3, 1:1 and 3:1. The results are shown in Table 4.

Table 4. Plasdone K-29/32 polymer is compatible in solutions and in films.

Compound	Solvent	% Total Solids	Solution Appearance	Film Compatibility
Carboxymethylcellulose (low viscosity grade)	Water	1	Soluble, clear, colorless	Compatible
Ethylcellulose	Ethanol	4	Partially soluble, cloudy, homogenous	Compatible
Methylcellulose	Water	-	Soluble	Compatible
Polyethylene Glycol	Ethanol	5	Soluble, clear, colorless	Compatible
Polyvinyl Alcohol	Water	-	Soluble	Compatible
Sodium Alginate	Water	-	Soluble	Compatible
Sodium Lauryl Sulfate	Water	5	Soluble, clear, colorless	Data Not Available

Crosslinking

Plasdone polymers can be crosslinked by treatment with persulfates, hydrazine, hydroperoxides or with gamma irradiation. When treated with water, the lightly crosslinked polymers will swell. These materials find use in wound care, medical adhesives and as lubricious coatings for medical devices, such as catheters.

Key Product Specifications & Typical Properties

Table 5 lists some of the key product specifications and typical properties. Full product specifications are available on request.

Table 5. Key Product Specifications and Typical Properties

	Plasdone							
	K-12	C-15	C-30	K-17	K-25	K-29/32	K-90	K-90D
Appearance	White to creamy white powder							
Appearance (5% aqueous)	Free of haze	Free of haze	Free of haze	Free of haze	Free of haze	Free of haze	Free of haze, colorless to pale yellow	Free of haze, colorless to pale yellow
Typical Tap Density (g/cm3)	0.54	0.60	0.47	0.60	0.49	0.47	0.46	0.53
Typical Bulk Density (g/cm3)	0.42	0.44	0.33	0.44	0.38	0.36	0.32	0.40
% Moisture	5.0 max	5.0 max	5.0 max	5.0 max	5.0 max	5.0 max	5.0 max	5.0 max
pH (5% aqueous solution)	3.0-5.0	3.0-5.0	3.0-5.0	3.0-5.0	3.0-5.0	3.0-5.0	4.0-7.0	4.0-7.0
% Ash	0.02 max	0.02 max	0.02 max	0.02 max	0.02 max	0.02 max	0.10 max	0.10 max
Vinyl Pyrrolidone, ppm	5.0 max	5.0 max	5.0 max	5.0 max	5.0 max	5.0 max	10.0 max	10.0 max
% 2-pyrrolidone	0.99 max	---	3.0 max	---	3.0 max	3.0 max	0.99 max	0.99 max
Heavy Metals, ppm (as lead)	10 max	10 max	5 max	10 max	5 max	5 max	5 max	5 max
% Aldehydes	0.05 max	0.05 max	0.05 max	0.05 max	0.05 max	0.05 max	0.05 max	0.05 max
%	11.5-	12.0-	12.0-	12.0-	12.0-	12.0-	12.0-	12.0-

Nitrogen	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8
K-Value (5% solids w/v aqueous solution)	10.2-13.8	15.5-17.5	29-32	16.0-17.5	24-26	29-32	85-95	85-95
Peroxide, ppm	400 max	400 max	400 max	400 max	400 max	400 max	400 max	400 max
Hydrazine, ppm	1.0 max	1.0 max	1.0 max	1.0 max	1.0 max	1.0 max	1.0 max	1.0 max
Pyrogen Endotoxin Units	---	0.5 max	0.5 max	---	---	---	---	---
Total Aerobic Plate Count, CFU/g	<100	<100	<100	<100	<100	<100	<100	<100
Mold/Yeast, CFU/g	<100	<100	<100	<100	<100	<100	<100	<100
Staphylococcus Aureus, CFU/g	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Salmonella, CFU/g	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Pseudomonas Aeruginosa, CFU/g	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
E. coli, CFU/g	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

EXHIBIT B

HERCULES

Physical and Chemical Properties

Aqualon

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KLUCEL® HYDROXYPROPYLCELLULOSE

CONTENTS PAGE

KLUCEL® HYDROXYPROPYLCELLULOSE

KLUCEL® hydroxypropylcellulose (HPC) is a nonionic water-soluble cellulose ether with a remarkable combination of properties. It combines organic solvent solubility, thermoplasticity, and surface activity with the aqueous thickening and stabilizing properties characteristic of other water-soluble cellulose polymers available from Hercules Incorporated, Aqualon Division. KLUCEL films are flexible without plasticizers and non-tacky at high humidity.

The information in this booklet presents the physical and chemical properties of KLUCEL as developed in our research and plant facilities. Also included is information about the behavior of KLUCEL with many of the materials that would be used in most applications. A guide to regulatory status and toxicological studies is provided for convenient reference. The Appendix gives information about Aqualon test methods for viscosity, moisture, and ash content.

To help the reader identify the versatile uses for this water-soluble polymer, a representative listing has been developed and is presented on the following page. Many of these uses for KLUCEL are discussed in detail in technical literature available from your Aqualon sales representative by request, and/or from our website at www.Aqualon.com.

APPLICATIONS

Applications for KLUCEL® Hydroxypropylcellulose

Types of Uses	Specific Applications	Properties Utilized
Adhesives	Solvent-based	Thickener
	Hot-melt	Thermoplastic
Aerosols	Food – whipped toppings	Stabilizer, foaming aid
	Emulsions – cosmetics	
	Solvent-based	Film-former, binder
Binder	Burnout types	Ready burnout, low residue, solvent-soluble
	Electrical insulators	
	Ceramic glazes	
	Alcohol core-wash compounds	Thickener, binder suspending agent
	Matrix board manufacture	Solvent-soluble
Coatings	Edible food coatings	Glaze, oil- and oxygen-barrier
	Textile and paper coatings	Solvent-soluble film-former, oil- and fat-barrier, heat-sealable
	Film coatings	
Cosmetics	Hair styling aids	Alcohol-soluble thickener and film-former
	Alcohol-based preparations	
	Perfumes and colognes	
	Emulsion creams, lotions, and shampoos	Emulsion stabilizer, thickener
Encapsulation	Micro- and macroencapsulation	Soluble, edible, flexible film barrier, fast release
Extrusion	Film and sheet	Binder, thermoplastic, water- and solvent-soluble
	Profiles and filament	
Foods	Whipped toppings	Stabilizer, whipping aid
	Edible coatings for nuts and candies	Protective coating and oil barrier
	Glaze for confections	High gloss and color coatings
	Fabricated foods	Binder for molding and extrusion
Molding	Injection-, compression-, and blow-molding	Binder, thermoplastic, water- and solvent-soluble
Paint removers	Acid-based	Thickener, acid resistant
	Scrape-off and flush-off	
Paper	Coatings	Solvent-soluble, flexible, thermoplastic film barrier
Pharmaceuticals	Tablet binder	Aqueous and solvent solubility
	Tablet coating	Thermoplastic binder
	Modified release	Non-ionic, pH insensitive
	Liquids and semisolids	Thickener, suspending agent, diffusion barrier
		Flexible films, surface active
Plastic foams	Foamed sheet, tube, rod	Thermoplasticity
Polyvinyl chloride processing	Suspension polymerization	Surface-active protective colloid
Printing	Inks (water-, alcohol-, and glycol-based)	Thickener, binder, suspending agent
Miscellaneous	Cleaners (acid-based)	Thickener, acid resistant
	Polishes (aqueous- and solvent-based)	Thickener, stabilizer, suspending agent

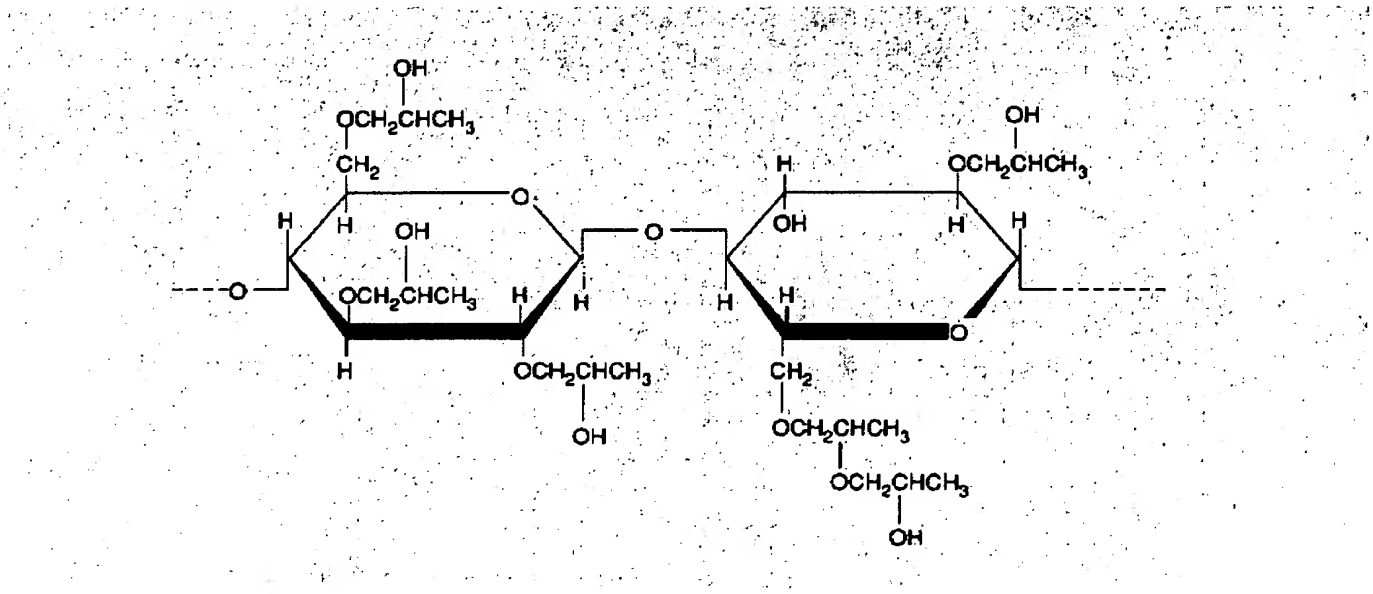
CHEMISTRY

KLUCEL® hydroxypropylcellulose is manufactured by reacting alkali cellulose with propylene oxide at elevated temperatures and pressures. The propylene oxide can be substituted on the cellulose through an ether linkage at the three reactive hydroxyls present on each anhydroglucose monomer unit of the cellulose chain. Published information suggests that etherification takes place in such a way that hydroxypropyl substituent groups contain almost entirely secondary hydroxyls. The secondary hydroxyl present in a side chain is available for further reaction with the oxide, and chaining-out may take place. This results in formation of side chains containing more than one mole of combined propylene oxide.

It is probable that most of the primary hydroxyls on the cellulose have been substituted and that the reactive groups remaining are secondary hydroxyls. Some typical molecular weight values are given in Table I.

An idealized structure for a portion of a hydroxypropyl-cellulose molecule with a molar substitution (MS) of 3.0 is given in Figure 1.

Figure 1
Structure of Hydroxypropylcellulose (MS 3.0)



GRADES AND VISCOSITY TYPES

KLUCEL® hydroxypropylcellulose is produced in several grades, determined by intended markets. For each grade, up to six viscosity types are available designated as H, M, G, J, L, E. Product designation is a combination of viscosity type followed by grade designation. For example: Klucel H Industrial, HF, H CS, and HF Pharm. Table I gives the water and ethanol viscosity specifications by grade for each available viscosity type.

Grades of KLUCEL

Intended Market	Grade Designation
Industrial	Industrial
Food	F
Personal Care	CS
Pharmaceutical	F Pharm

Table I – KLUCEL Viscosity Types, Viscosities (cps)[a], and Their Corresponding Molecular Weights

Industrial Grade	**Concentration in Water by Weight, %**				
Viscosity Types	1	2	5	10	Mw[b]
H Industrial	1,275-3,500	–	–	–	1,150,000
M Industrial	–	3,500-7,500	–	–	850,000
G Industrial	–	125- 450	–	–	370,000
J Industrial	–	–	125-450	–	140,000
L Industrial	–	–	65-175	–	95,000
E Industrial	–	–	–	250-800	80,000
Food Grade, Personal Care Grade, Pharmaceutical Grade					
Viscosity Types	1	2	5	10	Mw[b]
H CS, HF Pharm	1,500-3,000	–	–	–	1,150,000
M CS, MF Pharm	–	4,000-6,500	–	–	850,000
GF, G CS, GF Pharm	–	150- 400	–	–	370,000
JF, J CS, JF Pharm	–	–	150-400	–	140,000
LF, L CS, LF Pharm	–	–	75-150	–	95,000
EF, E CS	–	–	–	200-600	80,000
EF Pharm	–	–	–	300-600	80,000

Personal Care Grade, Pharmaceutical Grade	**Concentration in Anhydrous Ethanol, by Weight, %**				
Viscosity Types	1	2	5	10	Mw[b]
H CS, HF Pharm	1,000-4,000	–	–	–	1,150,000
M CS, MF Pharm	–	3,000-6,500	–	–	850,000
G CS, GF Pharm	–	75- 400	–	–	370,000
J CS, JF Pharm	–	–	75-400	–	140,000
L CS, LF Pharm	–	–	25-150	–	95,000
E CS, EF Pharm	–	–	–	150-700	80,000

[a] All viscosities are determined at 25°C using a Brookfield LVF viscometer with spindle and speed combinations depending on viscosity level. See Appendix.
[b] Weight-average molecular weight determined by size exclusion chromatography.

PROPERTIES

All grades conform to the specifications given in Table II. Typical properties are given in Table III.

Table II – Product Specifications for KLUCEL® HPC

Viscosity	Values are shown in Table I.
Physical form	White to off-white, granular solid
Particle size[1]	min. 85% through 30 mesh min. 99% through 20 mesh Industrial Grade: min. 80% through 30 mesh min. 98% through 20 mesh
Ash content, calculated as Na_2SO_4, %	0.2 max, silica free (No ash specification for Industrial Grades)
Moisture content (as packed), %	5.0 max
pH in water solution	Food and Personal Care Grades: 5.0-8.0 Pharmaceutical Grade: 5.0-7.5
Moles of substitution	3.4 to 4.4

[1]Fine x-grind Pharmaceutical Grade E and H viscosity types are available and designated as EXAF Pharm and HXAF Pharm. Particle size specifications are:
min. 80% through 100 mesh
min. 90% through 80 mesh
min. 99.9% through 60 mesh

MOISTURE ABSORPTION

KLUCEL absorbs moisture from the atmosphere, as do other water-soluble materials. The amount absorbed depends on relative humidity and temperature of the environment. As packed, moisture content of all grades does not exceed 5% by weight, and is generally between 2% and 3%. It is suggested that KLUCEL be stored in tightly closed containers and in a dry atmosphere to prevent any increase in moisture content.

KLUCEL has a low affinity for water. At any given relative humidity (RH), it has a lower equilibrium moisture content than most other water-soluble polymers. Typical values for KLUCEL are given below.

Equilibrium moisture content at 50% RH and 73°F (23°C)	4%
Equilibrium moisture content at 84% RH and 73°F (23°C)	12%

POLYMER STABILITY

Long-term storage stability of KLUCEL hydroxypropylcellulose is affected by the initial molecular weight of the polymer and storage conditions. Studies have shown that low- and medium-viscosity types maintain an average of 97% of their original viscosity after three years when stored at room temperature with frequent exposure to the atmosphere.

H viscosity types have a higher molecular weight and, therefore, are more susceptible to viscosity loss over time. Studies have shown that H viscosity types may sustain up to 10% viscosity loss after one year and 20% viscosity loss after two years. Storage at room temperatures with infrequent exposure to the atmosphere improves viscosity stability. Customers are encouraged to retest H viscosity types after one year and quarterly thereafter to assure material viscosity.

Table III – Typical Properties for KLUCEL

Product as Shipped	
Solubility in water[a]	Solutions are clear and smooth at temperatures below 38°C.
Solubility in organic solvents	Dissolves easily in many polar solvents to give clear, smooth solutions
Bulk density, g/ml	0.5 (varies with type)
Softening temperature	100-150°C
Burnout temperature	Burns out completely at 450° to 500°C in N_2 or O_2
Biological Oxygen Demand	14,000 ppm
Solutions in Water	
Specific gravity, 2% solution at 30°C	1.010
Refractive index, 2% solution	1.337
Surface tension, at 0.1%	43.6 dynes/cm
Interfacial tension, 0.1% KLUCEL in water vs refined mineral oil	12.5 dynes/cm
Bulking value in solution,	0.04 (0.334) gal/lb (l/kg)

[a]Silicon dioxide is added as an anticaking agent and may contribute a slight haze.

BURNOUT TEMPERATURE

KLUCEL has excellent binding properties. It is often used as a temporary binder in production of ceramics, glazes, refractories, and powdered metal products. This polymer is vaporized, or burned out, over the temperature range of 250° to 500°C in oxidizing, reducing, or inert atmospheres. The very low ash content of the original KLUCEL and the complete absence of organic residues after firing ensure virtually uncontaminated end products after burnout.

DISPERSION AND DISSOLUTION

KLUCEL® HPC is soluble in water at room temperature. It is insoluble in water above 45°C. It is readily soluble in many organic solvents, hot or cold. The best methods for preparing solutions of KLUCEL in water or organic solvents are described in the following paragraphs.

Note: As a general aid to preparation of solutions, the following points should be kept in mind:

- Wherever possible, KLUCEL should be put into solution before adding other soluble ingredients. Other dissolved materials compete for the solvent and slow the solution rate of KLUCEL. In this regard, soft water is preferred to hard water for solution preparation.
- KLUCEL is less soluble in hot water than in cold. In organic solvents, application of heat speeds the solution rate.

IN WATER
Most soluble polymers have a tendency to agglomerate, or lump, when the dry powder is first wet with solvent. Hydration of the outer surface of a particle, or an agglomerate of particles (lump), results in the formation of a viscous gel layer that inhibits wetting of the inside materials. The faster the rate of hydration of the polymer, the more quickly the gel layer will be developed, and the greater the tendency for lumping as the dry powder is added to the solvent.

KLUCEL hydrates somewhat slowly, and lumping can be avoided during solution preparation if the recommended procedures are followed. Lump formation should be avoided, as this can greatly increase the time required to prepare homogeneous solutions.

To prepare lump-free, clear solutions of KLUCEL in the shortest time, the following methods are suggested.

Method 1
The preferred method involves pre-slurrying the powder in a nonsolvent, such as hot water or glycerin, prior to addition to the main volume of water.

In the first step, prepare a high-solids slurry by adding dry KLUCEL powder to 6 times (or more) its weight of well-agitated hot water at a temperature of 50° to 60°C. Temperature should not exceed the 60°C maximum indicated. The hot slurry must be maintained above 50°C during this presoak to ensure that there is no premature dissolving of the particles that would result in the formation of a gelatinous mass. The slurry should be allowed to stir for a few minutes before addition to the main volume of cold water. This presoak results in a faster dissolving of particles in the second step.

In the second step, the hot slurry is diluted with cold water (room temperature or lower). Agitation is continued until all particles are dissolved and solution is completely free of gels. High-shear agitation is not necessary, and may be undesirable because of the tendency for foaming and air entrainment. In this dissolving step, the time factor is more important than high shear when it comes to ensuring complete solution of all gel particles.

Dissolving periods of 10 minutes or more may be required, depending on solution concentration and viscosity type being used. Solutions of lower-viscosity KLUCEL types at low-solids concentration require the shortest time for preparation.

Method 2
Add powdered KLUCEL to the vortex of well-agitated water at room temperature. The rate of addition must be slow enough to permit particles to separate in the water. Addition of the powder should be completed, however, before any appreciable viscosity buildup is obtained in the solution. The rate of agitation then may be reduced, but continued until a gel-free solution is obtained. Throughout the mixing period, solution temperature should be maintained below 35°C.

Method 3
Dry-blend KLUCEL with any inert or nonpolymeric soluble material that will be used in the formulation. Blending aids separation of particles of KLUCEL at first wetting and reduces the tendency to lump. For best results, KLUCEL should be less than 20% of the total dry blend. This blend is then handled as described in Method 2.

IN ORGANIC LIQUIDS
All types of KLUCEL have excellent solubility in a wide range of polar organic liquids and give clear, smooth solutions at ambient or elevated temperatures. There is no tendency for precipitation of KLUCEL in hot organic solvents; this is in contrast to its behavior in water solutions. Generally, the more polar the liquid, the better the solution. Methyl and ethyl alcohol, propylene glycol, dioxane, and Cellosolve are some of the best organic solvents for all types of KLUCEL.

Table VIII, page 15, lists the type of solutions obtained with G viscosity types in many organic liquids. The molecular weight of the type of KLUCEL can have a marked effect on solution quality in an organic liquid that is a borderline solvent for KLUCEL.

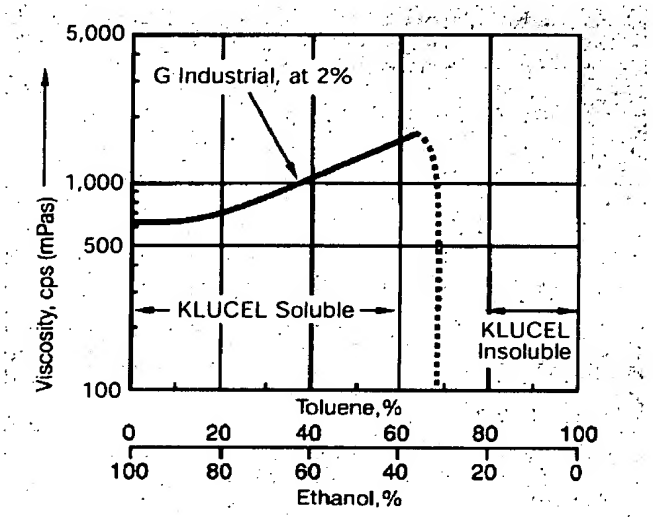
G viscosity types are intermediate in viscosity (i.e., molecular weight) between high H viscosity types and very low E viscosity types. In comparison with G viscosity types, lower-viscosity types are more readily soluble and may give better solutions. The higher-viscosity types may give slightly inferior solutions in some of the liquids listed in the table. For example, acetone gives excellent solutions with the E viscosity types, but acetone solutions of the G viscosity types are hazy and somewhat granular.

Solution quality in borderline solvents often can be greatly improved through the use of small quantities of cosolvents. Water, methanol, and ethanol function as excellent cosolvents and, in many cases, are effective in relatively small quantities (5% to 15%). For example, methylene chloride is a borderline solvent for high H viscosity types, and solutions are granular. Addition to the system of 10% methanol results in a smooth solution of normal viscosity (5,000 cps at 1% solids).

Elevated temperatures will improve solvent power of organic liquids for KLUCEL® HPC, and there is no tendency for precipitation of the polymer at these elevated temperatures. Heating of solvents will (a) reduce viscosity, (b) increase rate of solution, and (c) improve solution quality in the case of borderline solvents.

Aliphatic and aromatic hydrocarbons and petroleum distillates are nonsolvents for KLUCEL. However, relatively large quantities of these nonsolvents can be tolerated in a solution if KLUCEL is first dissolved in a solvent that is miscible with these nonsolvents. Examples of solvent-nonsolvent systems are given in Table VIII. Figure 2 details the effect of solvent composition on viscosity of a solution of G Industrial in a toluene:ethanol system.

Figure 2
Viscosity of G Industrial Dissolved in Toluene-Ethanol



In general, principles discussed for preparing water solutions apply when using organic solvents to make solutions of KLUCEL. Methods 1, 2, and 3 described for preparing water solutions can therefore be used to prepare solutions of KLUCEL in organic solvents. The "pre-slurry" principle of Method 1 can be employed through the use of nonsolvents such as glycerin, aliphatics, aromatics, and others.

In aqueous-organic systems, the proportion of organic solvent will determine whether elevated temperature will speed or slow the rate of solution of KLUCEL. (See Viscosity and Precipitation Temperature in Aqueous Alcohols, page 17).

IN HOT-MELTS AND WAXES

At elevated temperatures, many waxes are sufficiently good solvents so that KLUCEL is readily dissolved by addition of the dry powder to the stirred molten wax. Examples of materials that are good solvents for KLUCEL at an elevated temperature are: acetylated monoglycerides (e.g., Myvacet 5-00 and 7-00 series); glycerides (e.g., Myverol 18-07); polyethylene glycols (e.g., Carbowax); polypropylene glycol, pine oil, and tall oil fatty acids.

KLUCEL is compatible with a number of high-molecular-weight, high-boiling waxes and oils, and can be used to modify the properties of these materials. The addition of KLUCEL to these systems will increase viscosity and improve hardness and crack resistance to coatings.

PROPERTIES OF SOLUTIONS

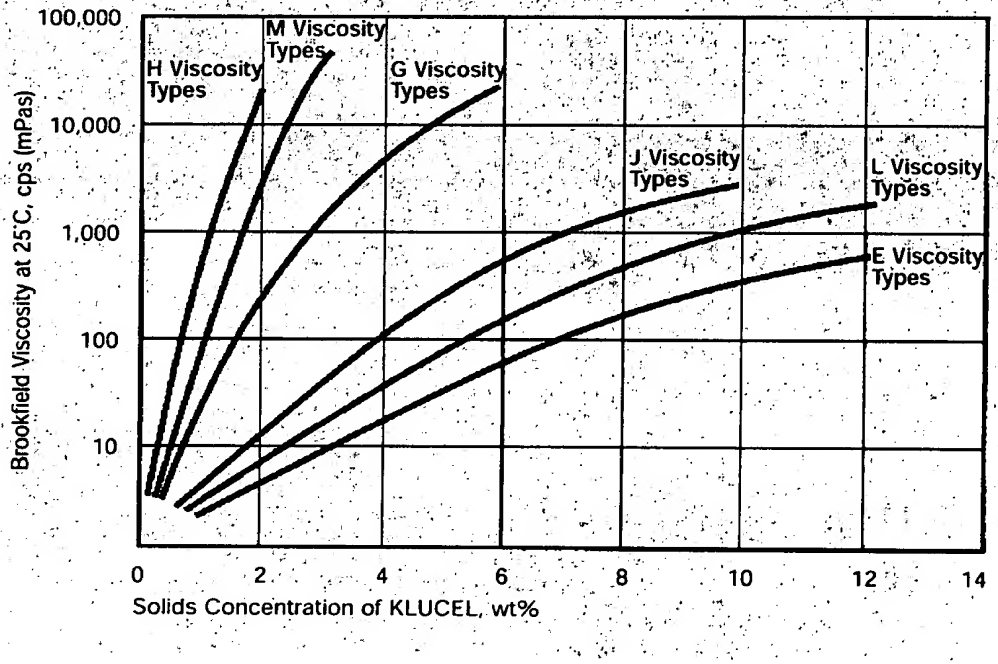
KLUCEL® HPC has excellent solubility in water and in many polar organic solvents, as discussed in the Dispersion and Dissolution section starting on page 7. Solutions are clear, smooth, and exceptionally free from gels and fibers. Solutions are non-Newtonian in flow, since they change in viscosity with rate of shear. But solutions display little or no thixotropy.

Because KLUCEL is used extensively to modify viscosity of solutions, dispersions, emulsions, and suspensions involving water and/or organic solvents, a discussion of some of the factors that affect solution viscosity follows.

AQUEOUS SOLUTIONS
At room temperature, solutions of KLUCEL can be prepared in a wide range of viscosities, depending on concentration and viscosity type used. Since solutions are non-Newtonian, it is essential to standardize viscosity determination methods. The method used in the control laboratory at Aqualon is described in detail in the Appendix.

Effect of Concentration and Viscosity Type
The viscosity of solutions of KLUCEL increases rapidly with concentration and becomes almost a straight-line relationship when plotted on a semi-log basis. (See Figure 3.) The bands in this figure indicate the viscosity range within which each type is supplied. (See also Table I, page 5.)

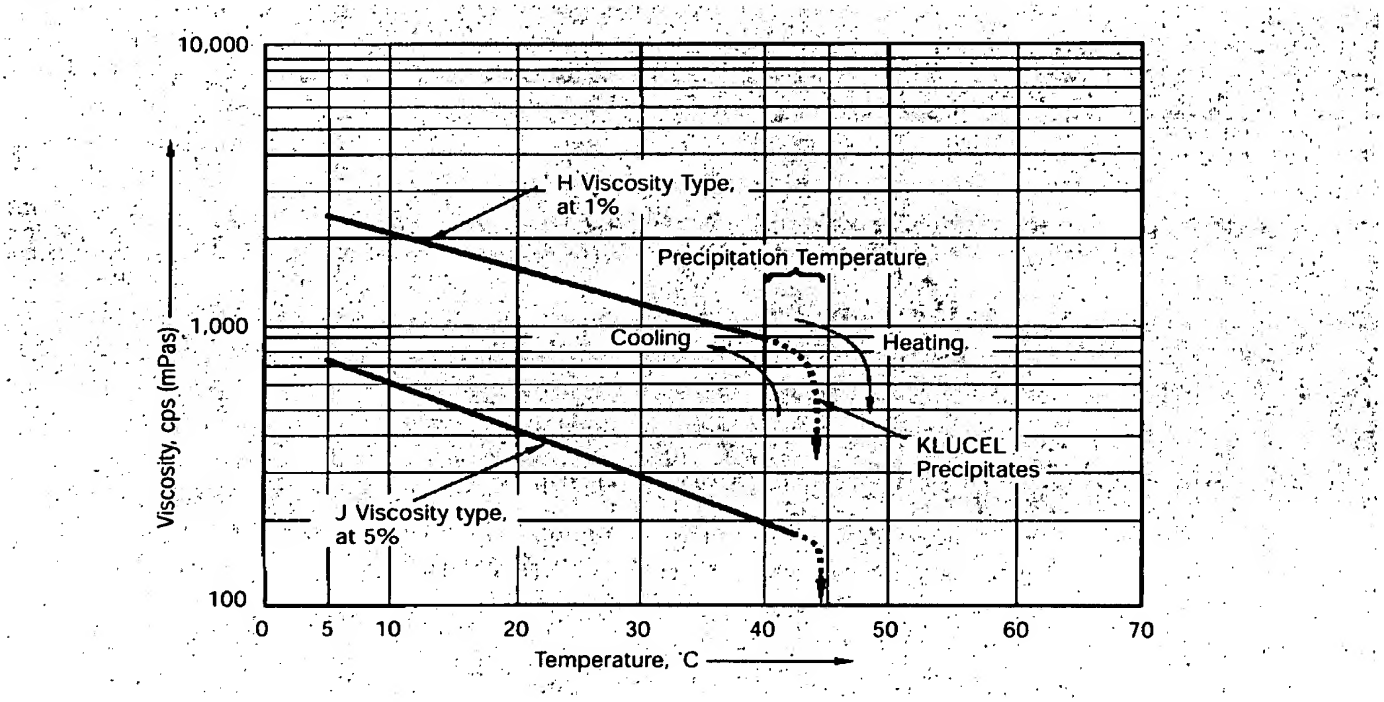
Figure 3
Effect of Concentration and Type of KLUCEL on Viscosity of Water Solutions



Rheology
Solutions of KLUCEL are exceptionally smooth-flowing and exhibit little or no structure or thixotropy. However, solutions are pseudoplastic under conditions of high rates of shear and will show a temporary decrease in viscosity while under shear. The viscosity returns to the original value when the shear is removed. The lower the molecular weight of KLUCEL and the lower the shear rate, the less will be this decrease in viscosity experienced under shear.

Effect of Temperature
Viscosity of an aqueous solution of KLUCEL decreases as temperature is increased. This effect is normal for polymers in solution. This change in viscosity is illustrated in Figure 4 for H viscosity type and J viscosity type. As shown, viscosity is halved as temperature is raised through 15°C. This effect is uniform up to the precipitation temperature (40° to 45°C).

Figure 4
Effect of Temperature on the Viscosity of Aqueous Solutions of KLUCEL



Precipitation Temperature in Water
As already stated, KLUCEL® HPC will precipitate from water solution at a temperature between 40° and 45°C. This precipitation is completely reversible. The polymer redissolves upon cooling the system below 40°C with stirring, and the original viscosity is restored.

As the temperature reaches 40° to 45°C, this precipitation phenomenon is evidenced by appearance of cloudiness in the aqueous solution and by a marked reduction in viscosity. These effects are due to separation of the polymer as a highly swollen precipitate. The transition from dissolved to precipitated polymer takes place without the formation of a gel. The only apparent viscosity change is one of a rapid decrease, as shown in Figure 4.

The form in which KLUCEL precipitates from aqueous solutions depends not only on the molecular weight of the polymer, but also on other materials present in solution and whether or not stirring is employed. Low-viscosity types tend to separate as highly swollen and finely divided precipitates. High-viscosity types, particularly under agitation, may agglomerate on heating and form a stringy, rather than a finely divided, precipitate. The addition of Aqualon® cellulose gum or surfactants reduces the tendency for agglomeration of the KLUCEL polymer as it precipitates, and by this means, the high-viscosity types also can be separated in finely divided form.

The precipitation temperature of KLUCEL is increased through the addition of organic liquids that are solvents for the polymer. The precipitation temperature in aqueous alcohols and aqueous glycol is discussed in another section.

The precipitation temperature is lower in the presence of relatively high concentrations of other dissolved materials that compete for the water in the system. The magnitude of the lowering is dependent on the nature and concentration of the other dissolved ingredients. The data in Table IV illustrate this effect.

Table IV – Comparative Effect of Solution Composition on Precipitation Temperature

Ingredients and Concentration	Precipitation Temperature, °C
1% H viscosity type	41
1% H viscosity type + 1.0% NaCl	38
1% H viscosity type + 5.0% NaCl	30
0.5% H viscosity type + 10% Sucrose	41
0.5% H viscosity type + 20% Sucrose	36
0.5% H viscosity type + 30% Sucrose	32
0.5% H viscosity type + 40% Sucrose	20
0.5% H viscosity type + 50% Sucrose	7

Compatibility With Surfactants
Compatibility of KLUCEL with surface-active agents will vary according to the particular agent and concentration, as well as to the grade and concentration of KLUCEL used.

Because of its hydroxypropyl substitution, KLUCEL is more lipophilic in nature than other water-soluble cellulose derivatives. Accordingly, it is compatible with a wide range of anionic, nonionic, cationic, and amphoteric surfactants.

Addition of Certain Ionic Surfactants
Studies with KLUCEL using certain ionic surfactants have aided the development of technology to permit thickening at temperatures in excess of the normal cloudpoints for KLUCEL. Aqueous solutions of M viscosity type and sodium lauryl sulfate, at a surfactant to KLUCEL ratio of 1:3 or greater, result in cloudpoints in excess of 70°C. At some ratios, cloudpoints greater than 95°C may be achieved.

Included in these studies were the following ionic surfactants, which proved to be effective:

- Sodium lauryl sulfates
- Ammonium lauryl sulfates
- Lauryl alcohol ether sulfate
- Trimethylcoco ammonium chloride

The nonionic surfactants studied were not effective in raising the cloudpoints.

Effect of pH
KLUCEL HPC is a nonionic polymer, and viscosity of water solutions is not affected by changes in pH. The viscosity of solutions remains unchanged as pH is varied over the range of 2 to 11.

However, where long-term storage stability is required, the solution pH is an important consideration because of degradation that can occur under highly acid or alkaline conditions, as described in the section titled Viscosity Stability, page 13.

Effect of Inorganic Salts
The compatibility of KLUCEL with dissolved inorganic salts in water solution varies according to the salt. If relatively high concentrations of dissolved salts are used, there is a tendency for the KLUCEL polymer to be "salted out" from solution as a finely divided and highly swollen precipitate.

This salting-out phenomenon generally results in some decrease in viscosity and in the appearance of cloudiness in the solution. In borderline cases, this salting out may not be immediately apparent, but may occur upon standing.

The compatibility of G viscosity types with a number of selected salts is illustrated in Table V.

Table V – Compatibility of KLUCEL® HPC With Some Inorganic Salts[a]

Salt	Salt Concentration, % by weight			
	2	5	10	50
Aluminum sulfate	C	I	–	–
Ammonium nitrate	C	C	C	I
Ammonium sulfate	C	I	–	–
Calcium chloride	C	C	C	I
Disodium phosphate	I	–	–	–
Ferric chloride	C	C	C	I
Potassium ferrocyanide	C	C	I	–
Silver nitrate	C	C	C	I
Sodium acetate	C	C	I	–
Sodium carbonate	C	I	–	–
Sodium chloride	C	C	I	–
Sodium nitrate	C	C	C	I
Sodium sulfate	C	I	–	–
Sodium sulfite	C	I	–	–
Sodium thiosulfate	C	I	–	–
Sucrose	C	C	C	I

Key: C = compatible; I = incompatible

[a] Tests were conducted by adding a 2% solution of G viscosity type to salt solutions of various concentrations. The salt concentration in the system is indicated, and the final concentration of KLUCEL was approximately 0.1% by weight in all cases.

Compatibility With Other Polymers
KLUCEL has a wide range of compatibility with organic materials. The dual solubility of KLUCEL permits its admixture with water-soluble, as well as solvent-soluble, resins, polymers, and organic liquids.

In spite of this wide compatibility, the KLUCEL polymer, when used in aqueous systems, may not tolerate high concentrations of other dissolved materials. The balance of hydrophilic-lipophilic properties of the polymer, which are required for dual solubility, reduces its ability to remain hydrated in the presence of high concentrations of other dissolved materials. KLUCEL may precipitate or "salt out" under these conditions.

A detailed discussion of compatibility with natural and synthetic polymers and inorganic salts follows.

Water-Soluble Polymers
KLUCEL is compatible with most natural gums and synthetic water-soluble polymers. Solutions in water are homogeneous, and films cast from these solutions are uniform. The following have been tested and found to be compatible:

- AQUALON® sodium carboxymethylcellulose (CMC)
- NATROSOL® hydroxyethylcellulose (HEC)
- BENECEL® methylcellulose (MC)
- SUPERCOL® guar gum
- Gelatin
- Sodium caseinate
- Polyethylene oxide
- Carbowax 1000
- Polyvinyl alcohol
- Sodium alginate
- Locust bean gum

The effect on solution viscosity of blends of KLUCEL and other water-soluble polymers varies, depending on the ionic nature of the copolymer. To illustrate, blends of KLUCEL with NATROSOL hydroxyethylcellulose and CMC were studied. The blends were prepared at a 1:1 ratio of the two polymers. The results of this study are given in Table VI.

Solution viscosity of blends of nonionic polymers KLUCEL and NATROSOL was essentially in agreement with the calculated value. This was true for all viscosity types studied.

When solutions of blends of KLUCEL and an ionic polymer, CMC, were prepared, the resultant viscosity was greater than the calculated value. The synergism of this combination increased with increasing molecular weight of the polymers.

The data shown in Table VI were obtained with solutions prepared in distilled water or tapwater. The synergistic effect may be drastically reduced in the presence of low levels of dissolved salts or if pH is below 3 and above 10.

Table VI – Blends of KLUCEL® HPC and Other Water-Soluble Polymers: Effect on Viscosity

Polymer Blend (1:1)	Concentration, %	Solution Viscosity, cps (mPas)		
Viscosity Types		Expected	Initial	After 24 Hours
J and NATROSOL® 250J HEC	5	235	240	235
M and NATROSOL 250M	2	6,250	5,900	5,600
H and NATROSOL 250H	1	2,320	2,440	2,440
H and AQUALON® CMC-7H	1	2,220	4,400	3,860

Water-Insoluble Polymers
KLUCEL is compatible with many natural and synthetic latexes available as emulsions in water. KLUCEL is soluble in the aqueous phase, and uniform films and coatings are obtained upon drying.

Using common solvents, KLUCEL has been incorporated with water-insoluble polymers such as zein, shellac, AQUALON ethylcellulose, and cellulose acetate phthalate. Films and coatings prepared from these systems are homogeneous and of good quality.

Viscosity Stability
Water solutions of KLUCEL possess best viscosity stability when pH is held between 6.0 and 8.0, and when the solutions are protected from light, heat, and action of microorganisms.

KLUCEL in water solution, like other water-soluble polymers, is susceptible to both chemical and biological degradation. This degradation generally results in reduction of molecular weight of the dissolved polymer, with an accompanying decrease in viscosity of the solution. Some loss of solution clarity may occur in cases of severe biological degradation.

KLUCEL has demonstrated greater resistance to chemical and biological degradation than other cellulose ethers. Techniques to minimize degradation mechanisms are discussed in the following two sections.

Hydrolysis and Oxidation
KLUCEL in solution is susceptible to acid hydrolysis, which causes chain scission and loss of viscosity of the solution. The rate of hydrolysis increases with temperature and hydrogen ion concentration. Solutions should be buffered to pH 6.0 to 8.0 and maintained at low temperature to minimize acid hydrolysis.

Alkali-catalyzed oxidative degradation will also degrade the polymer and result in decrease in viscosity of solution. The degradation can result from presence of dissolved oxygen or oxidizing agents in the solution. Peroxides and sodium hypochlorite under alkaline conditions cause rapid degradation. For best stability on storage, pH should be maintained between 6.0 and 8.0 and antioxidants should be used if oxidative degradation is likely to occur.

Ultraviolet light will degrade the cellulose, and solutions of KLUCEL will undergo some decrease in viscosity if exposed to light for several months.

Biological Stability
The high level of substitution of KLUCEL improves resistance of this polymer to degradation by cellulase enzymes produced by molds and bacteria. However, water solutions are susceptible to degradation under severe condtions, and a viscosity decrease may result. If prolonged storage is contemplated, a preservative is recommended.

Certain enzymes, produced by microbial action, will degrade KLUCEL in solution. If microbial contamination is present in makeup water, it is important that sterilization techniques effective against enzymes as well as against microorganisms be employed prior to preparing the solution of KLUCEL.

Solutions of KLUCEL in organic solvents do not generally require preservative.

Preservatives
Listed below are some of the preservatives that are effective in preserving solutions of KLUCEL. It is recommended that the preservative manufacturer be consulted regarding kind, amount, and rate of use for the preservative to be added.

- Dowicil 100 n-(3-chloroallyl)-hexaminium chloride
- Formaldehyde
- Phenol
- Omadine

Some of the preservatives that are effective with KLUCEL and are in compliance with the Food and Drug Administration for use in food are listed below.

- Sodium benzoate
- Sorbic acid and its potassium, sodium, and calcium salts
- Sodium propionate
- Methyl and propyl parahydroxybenzoate

Note: Solutions of KLUCEL have demonstrated some incompatibility with a number of preservatives based on substituted phenol derivatives.

Surface and Interfacial Tension
KLUCEL® HPC is a surface-active polymer. Water solutions display greatly reduced surface and interfacial tension. Because of this, KLUCEL functions as an aid in both emulsifying and whipping. These properties, coupled with protective colloid action, enable it to perform dual functions in the following systems:

- Oil-in-Water Emulsions – Stabilizer and emulsification aid
- Foamed Systems – Stabilizer and whipping aid

The reduction in surface and interfacial tension of water solutions containing KLUCEL is illustrated in Table VII. All viscosity types have essentially the same effects, and a concentration of KLUCEL as low as 0.01% produces close to the maximum reduction in surface tension.

Recommended Defoamers
The low surface tension of water solutions containing KLUCEL tends to promote foaming and air entrainment. If this presents a difficulty, a water-dispersible antifoam agent can be used and should be added to the water prior to solution preparation.

Water-dispersible defoamers such as HERCULES® 1512 defoamer; Colloid 581-B; Nopco NDW and KFS; Antifoam AF; or lauryl or octyl alcohol are effective. Defoamed concentrations generally run 25 to 200 ppm, but it is suggested that the manufacturers be consulted for their recommendations for the particular system involved.

Table VII – Surface and Interfacial Tensions of KLUCEL Solutions at 25°C

KLUCEL, wt%	Surface Tension, dynes/cm (mN/m)	Interfacial Tension vs Refined Mineral Oil, dynes/cm (mN/M)
0 (water)	74.1	31.6
0.01	45.0	–
0.1	43.6	12.5
0.2	43.0	–

VISCOSITY IN ORGANIC SOLVENT SOLUTIONS

The viscosity-vs-concentration curve for KLUCEL® HPC dissolved in organic liquids, which are good solvents for the polymer, shows the same general pattern as that for KLUCEL dissolved in water (Figure 3, page 9). The viscosity rises rapidly as the concentration of polymer is increased. The curves for viscosity in ethanol and methanol parallel those for viscosity in water, but are displaced toward somewhat lower viscosity values.

Table VIII – Solvents for KLUCEL[a]

A. CLEAR AND SMOOTH

Acetic acid (glacial)	Isopropyl alcohol (95%)
Acetone:water (9:1)	Methanol
Benzene:methanol (1:1)	Methyl Cellosolve
Cellosolve	Methylene chloride: methanol (9:1)
Chloroform	Morpholine
Cyclohexanone	M-Pryol
Dimethyl formamide	Propylene glycol
Dimethyl sulfoxide	Pyridine
Dioxane	t-Butanol:water (9:1)
Ethyl alcohol	Tetrahydrofuran
Ethylene chlorohydrin	Toluene:ethanol (3:2)
Formic acid (88%)	Water
Glycerin:water (3:7)	

B. MODERATELY GRANULAR AND/OR HAZY

Acetone	Methyl acetate
Butyl acetate	Methyl ethyl ketone
Butyl Cellosolve	Methylene chloride
Cyclohexanol	Naphtha:ethanol (1:1)
Isopropyl alcohol (99%)	Tertiary butanol
Lactic acid	Xylene:isopropyl alcohol (1:3)

C. INSOLUBLE

Aliphatic hydrocarbons	Methyl chloroform
Benzene	Mineral oils
Carbon tetrachloride	Soybean oil
Dichlorobenzene	Toluene
Kerosene	Gasoline
Trichloroethylene	Glycerin
Xylene	Linseed oil

[a] Solvents were tested using G viscosity types at 2% solids concentration by weight. All ratios indicated in this table are on a by-weight basis.

Where organic liquids that are borderline solvents for KLUCEL® HPC are used, unusual viscosity effects can be observed. Viscosity may be abnormally high or abnormally low, depending on the degree of solvation of the polymer. For example, as shown in Table IX, H viscosity types in methylene chloride gave a poor solution with reduced viscosity. The L viscosity types, which was better solvated, gave a solution with unusually high viscosity. In both cases, the addition of a small amount of a cosolvent (10% methanol) gave solutions with normal viscosities.

Table IX lists typical solution viscosities for various types of KLUCEL in a number of solvents.

Table IX – Comparative Viscosity of KLUCEL in Water and Certain Organic Solvents

Solvent	Viscosity[a] Type of KLUCEL and Concentration, cps (mPas)			
	H at 1%	G at 2%	L at 9%	E at 10%
Water	2,100	270	80	275
Methanol	800	85	25	75
Methanol:water (3:7 by weight)	–	360	–	–
Ethanol	1,600	210	65	255
Ethanol:water (3:7)	–	500	–	–
Isopropyl alcohol (99%)	[b]	[b]	145	570
Isopropyl alcohol (95%)	–	–	130	420
Acetone	[b]	[b]	50	175
Methylene chloride	4,500[b]	–	1,240[b]	14,600[b]
Methylene chloride:methanol (9:1)	5,000	–	400	–
Chloroform	–	–	2,560[a]	17,000[b]
Propylene glycol	6,000	6,640	5,020	>10,000
Ethylene chlorohydrin	470	430	310	1,110

[a] Viscosities shown are presented only as typical values. Some variation in these viscosities will be obtained from lot to lot of each type of KLUCEL.
[b] Borderline solvent for the particular type of KLUCEL. Solutions are granular and may be hazy.

Viscosity and Precipitation Temperature in Aqueous Alcohols

The viscosity of solutions of KLUCEL® HPC in aqueous alcohols varies with composition of the solvent. The viscosity goes through a maximum value at a solvent composition of 7 parts water: 3 parts alcohol by weight. This is illustrated in Figure 5. This type of viscosity curve is obtained when KLUCEL is added directly to aqueous alcohol or when it is first dissolved in either water or alcohol with subsequent addition of the second solvent.

Addition of alcohol to a water solution of KLUCEL will increase the temperature at which the polymer will precipitate from solution. Temperature elevation is dependent on type and concentration of alcohol. The effect obtained with methanol and ethanol is detailed in Figure 6. As shown, solutions of KLUCEL containing 45% (by volume) of ethanol or methanol can be heated to the boiling point of the solution without precipitation of KLUCEL.

Propylene glycol performs similarly to methanol, and elevation of precipitation temperature falls on the same curve. Other water-miscible organic liquids, which are good solvents for KLUCEL, will also function to elevate precipitation temperature of the polymer in the system.

Figure 5
Viscosity of Aqueous Alcohol Solutions (G viscosity types at 2% concentration by weight)

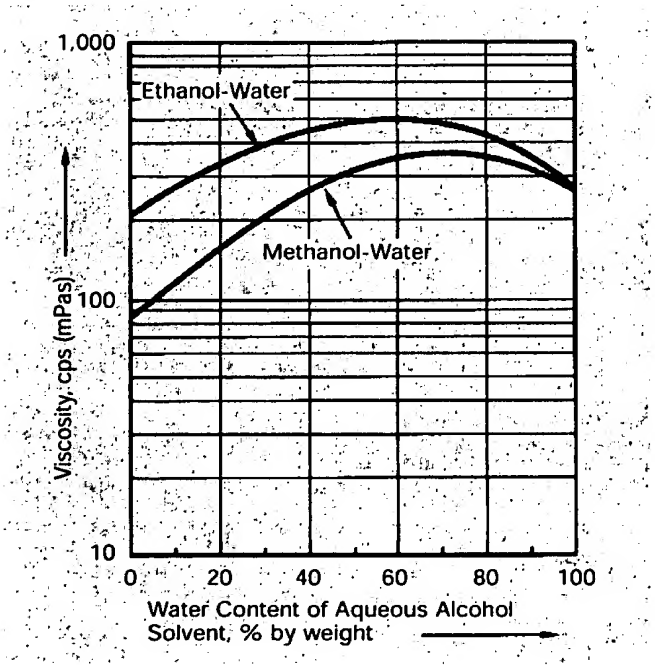
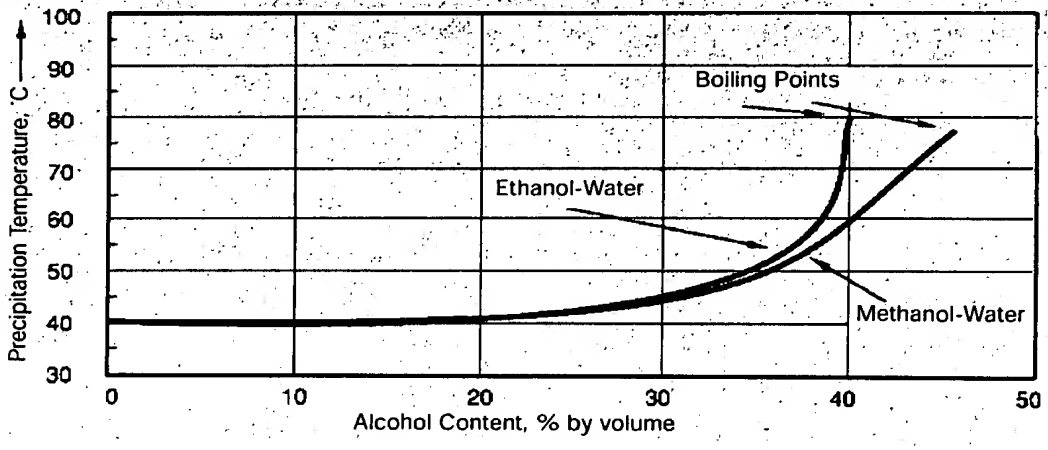


Figure 6
Precipitation Temperature of KLUCEL in Aqueous Alcohols



THERMOPLASTICITY – MOLDING AND EXTRUSION

KLUCEL® HPC is an excellent thermoplastic polymer that can be processed by virtually all fabrication methods used for plastics. Injection- and compression-molding; blow-molding; injection foam molding; vacuum-forming; and extrusion of film, sheet, foam profiles, and filament have been demonstrated on conventional plastics equipment, using pelletized molding powder.

All molecular weight types are thermoplastic and can be readily molded and extruded. In general, the low-molecular-weight types are preferred in injection- and blow-molding, where rigidity, hardness, and dimensional stability are most important. The medium- to high-molecular-weight types are recommended for most extrusion systems where greater flexibility and higher tensile properties are desired. When fillers or extenders are used, the higher-molecular-weight types of KLUCEL are selected for their superior binding properties and their ability to maintain good flexibility and toughness, even at filler levels up to 90% by weight.

FORMULATION OF MOLDING COMPOUNDS

While not essential, small amounts of plasticizers are normally used with KLUCEL to ensure smooth, uniform melt flow and homogeneous end products. The addition of plasticizers will give finished articles softer and more flexible characteristics. Plasticizers such as propylene glycol, glycerin, polyethylene glycols, and trimethylolpropane are generally suitable.

Internal lubricants are added to KLUCEL molding powders to provide lubrication at the die wall or mold wall, ensuring easy release from mold surfaces and preventing lamination of the KLUCEL to itself, as in a roll of KLUCEL hydroxypropyl-cellulose film. Glycerol monostearate, silicones, lecithin, and various stearates have proved useful.

Antioxidants also can be added to the low-molecular-weight types to prevent discoloration and thermal degradation. Butylated hydroxytoluene, lauryl thiodipropionate, and ascorbic acid are a few of the effective antioxidants commonly used.

Fillers, extenders, and compatible polymers may be added to KLUCEL to impart some functional property, to vary physical characteristics, or to reduce costs. In general, any filler material that will withstand the melt temperatures of KLUCEL can be incorporated and processed as with other plastics. Numerous filler materials, such as starch, talc, detergents, fragrances, pigments, fertilizers, various food products, and some drugs and medicinals, have been successfully evaluated in molding and extrusion systems. Depending on the particle size and bulk density of the filler, loadings of 40 to 95 weight % are possible with KLUCEL.

A twin-screw extruder is recommended for the preparation of pelletized molding powders. A single-screw extruder can be used if it is equipped with a nylon screw or metering screw having a long, deep-flighted feed section.

Most articles fabricated from KLUCEL are custom-formulated to obtain the desired physical properties and solubility rate required for a specific application. We suggest that you contact your Aqualon sales representative for assistance in formulating a product to meet your needs.

FILMS AND COATINGS

The excellent film properties of KLUCEL® HPC make it a useful material for fabrication of film and sheet. It is also useful in the formulation of coatings on paper, fabrics, food products, and other substrates. Flexible packaging films and sheet can be produced with conventional extrusion techniques. Coatings can be applied by extrusion or from aqueous or organic solvent systems.

FILM PROPERTIES
All films of KLUCEL are characterized by the following outstanding properties:

- Excellent flexibility
- Lack of tackiness at high humidity
- Good heat sealability
- Barrier to oil and fat

PLASTICIZERS FOR FILMS
Since films of KLUCEL® HPC are inherently flexible, it is not necessary to add plasticizers to cast films. However, in extruded films, plasticizers provide desirable die lubrication, reduction in melt viscosity, and improvement in melt uniformity. In all films of KLUCEL, added plasticizer will soften the film, reduce heat-seal temperatures, and impart improved flexibilty and increased elongation. Plasticizer levels of 5% or less (usually 2% or less) are recommended. Higher addition levels lead to reduced tensile properties, increased tackiness at high humidity, and poorer clarity, dimensional stability, and solubility characteristics.

The following plasticizers are compatible in films of KLUCEL at a concentration of 5%:
- Propylene glycol
- Glycerin
- Trimethylolpropane
- Polyethylene glycols

For improved resistance to tackiness at high humidity, the following lubricants are recommended:
- Polyethylene glycols (1,000 to 20,000 molecular weight)
- Propylene glycol monostearate
- Glycerol monostearate
- Silicone

ADDITIVE AND MODIFIER FOR FILMS AND COATINGS
The good film properties and wide range of solubility of KLUCEL make it a useful additive and modifier for other films and coatings. Solubility in polar organic solvents permits addition to many organic-solvent-soluble resins, while a broad range of compatibility ensures homogeneous films of improved quality.

The addition of KLUCEL in coatings generally:
- Improves flexibility.
- Improves toughness.
- Improves heat-seal properties.
- Reduces water resistance.
- Reduces the tendency to crack.

SOLUTION CASTING FOR FILMS AND COATINGS
The solubility of KLUCEL in water as well as in polar organic solvents affords a wide choice in the preparation of solutions used for casting film or for coating operations.

KLUCEL is insoluble in hot water, so care must be exercised when drying aqueous coatings to avoid the "blushing" that can occur at high temperatures and that might impair the film properties.

INSOLUBILIZING FILMS AND COATINGS
Films and coatings of KLUCEL can be made insoluble through crosslinking with resins that are reactive with the available hydroxyl groups on the KLUCEL polymer. A number of resins are capable of performing this crosslinking reaction.

Rate of cure and extent of insolubility of the final composition are generally dependent not only on the reactivity of the insolubilizing resin, but also on the temperature of the cure, pH of the system, and the amount of crosslinking resin used. High temperature, low pH, and higher proportions of resins tend to increase the rate of cure, improve water resistance, and increase stiffness.

The crosslinked composition may possess a high degree of insolubility in water, but the composition is not completely resistant and generally swells in the presence of water. This means that films and coatings have reduced wet strength and lose toughness.

PACKAGING, REGULATORY STATUS, TOXICOLOGY, SAFETY

PACKAGING
KLUCEL® HPC is shipped in fiber drums with a polyethylene inner liner containing 100 lbs net.

REGULATORY STATUS
Food grade, designated with a "F," complies with the requirements of the US FDA for direct addition to food for human consumption, as specified in the Code of Federal Regulations, Title 21, Section 172.870. Food grade Klucel also conforms to the specifications for hydroxypropylcellulose set forth in the *Food Chemicals Codex.*

Personal Care grade, designated with "CS," conforms to the specifications for hydroxypropylcellulose set forth in the CTFA.

Pharmaceutical grade, designated with "F Pharm," conforms to the specifications for hydroxypropylcellulose set forth in the *U.S. National Formulary, European Pharmacopoeia* and the *Japanese Pharmacopoeia.*

TOXICOLOGY
Toxicity testing indicates that KLUCEL is physiologically inert. The results of repeat-insult patch tests on humans disclose no evidence that it is either a primary skin irritant or skin-sensitizing agent. Feeding of KLUCEL to rats for 90 days at dietary levels up to and including 5.0% produced no gross or histopathologic changes or other deleterious effects. KLUCEL is considered nutritionally equivalent to purified cellulose in that neither material is metabolized. For a complete report, request a copy of Bulletin T-122, "KLUCEL® Hydroxypropylcellulose: Summary of Toxicological Investigations."

PRODUCT SAFETY
KLUCEL is a flammable dust when finely divided and suspended in air. An explosion can occur if the suspended dust is ignited. Proper design and operation of facilities and good housekeeping practices can minimize this hazard.

Floors subject to spills or dusting with KLUCEL can become slippery when wetted with water. Follow good housekeeping practices and clean up spills promptly.

Read and understand the Material Safety Data Sheet (MSDS) before using this product.

CASRN:	9004-64-2
CAS Name:	Cellulose 2-hydroxypropyl ether

APPENDIX

METHODS OF ANALYSIS
All types of KLUCEL® HPC meet certain specifications for moisture, viscosity, and ash content. Detailed descriptions are given of Aqualon methods for determining these values.

A. Moisture

1. Weigh duplicate samples of 5 g, to the nearest 0.001 g, into previously dried and weighed moisture cans with covers.
2. Place the samples in a gravity convection oven maintained at 105° ± 0.5°C. Heat for 3 hrs. Cool in a desiccator and weigh.
3. Return the sample to the oven for 45 min. Cool and weigh as before. If the second dried weight is not within 0.005 g of the first dried weight, repeat the 45-min oven periods until two subsequent weighings are in agreement. Then, using the lowest dried weight obtained, calculate percent moisture as follows:

$$\frac{\text{Original sample weight} - \text{dry sample weight}}{\text{Original sample weight}} \times 100 = \% \text{ moisture}$$

B. Viscosity in Water
As explained on page 9, the apparent viscosity of a solution of KLUCEL depends on a number of factors. If reproducible results are to be obtained, a closely standardized method of solution preparation and viscosity determination must be followed.

Preparation of the solution is critical in that KLUCEL must be completely dissolved in order to obtain a significant measurement. In weighing out the proper amount of KLUCEL for a viscosity determination, care must be taken to include a moisture correction. This correction compensates for the moisture in KLUCEL and places the viscosity measurement of KLUCEL on a dry basis. The viscosity-measurement test must be rigidly standardized because the viscosity reading obtained is dependent on the rate of shear during dissolution, the amount of agitation prior to measurement, the temperature, and the elapsed time between agitation and measurement. The method used in Aqualon laboratories is therefore given here in detail.

Solution Preparation
Immediately after taking portions of the KLUCEL polymer sample for moisture determination, portions of the same undried KLUCEL should be taken for viscosity solution preparation. Weighings of moisture and solution samples should be carried out practically together to ensure that the moisture content of the respective portions is the same at time of weighings.

The standardized Aqualon method for determining the viscosity of solutions of KLUCEL specifies use of the Brookfield viscometer, Model LVF (Brookfield Engineering Labs, Stoughton, Massachusetts; 4 spindles, 4 speeds covering the range 0 to 100,000 cps).

The solution volumes specified under item 4 should not be less than outlined or there may not be sufficient solution to cover the appropriate Brookfield spindle.

1. Weigh the specified amounts to ± 0.005 g, as obtained from Table XIII, page 24, quickly into clean, ground-glass-stoppered weighing bottles. Stopper immediately to eliminate gain or loss of moisture content.
2. From the determined percent moisture, calculate the water to be added for the respective viscosity solutions, as follows:

   a. For a 1% viscosity solution:

   $$(\text{Weight of KLUCEL, g}) \times (99 - \%\ \text{moisture}) = \text{Weight of water, g}$$

   b. For a 2% viscosity solution:

   $$\frac{(\text{Weight of KLUCEL, g}) \times (98 - \%\ \text{moisture})}{2} = \text{Weight of water, g}$$

   c. For a 5% viscosity solution:

   $$\frac{(\text{Weight of KLUCEL, g}) \times (95 - \%\ \text{moisture})}{5} = \text{Weight of water, g}$$

   d. For a 10% viscosity solution:

   $$\frac{(\text{Weight of KLUCEL, g}) \times (90 - \%\ \text{moisture})}{10} = \text{Weight of water, g}$$

3. Add the calculated amount of distilled water to a 16-oz bottle.
4. Stir the water with a mechanical agitator to create a vortex, and slowly sift the KLUCEL into the vortex over a 15 to 30-sec period to ensure good dispersion. An anchor-shaped stirrer turned by a compressed-air or electric motor has been found to be satisfactory. After the solution appears to be complete, stir it for an additional 10 to 15 min at high speed. Be careful to avoid loss of solution.
5. When the solution is complete, cover the mouth of the bottle with cellophane and screw the cap on securely. Place it in a constant-temperature bath (25° ± 0.2°C) for 30 min, or for as long as necessary to adjust the solution temperature to 25° ± 0.2°C.

Viscosity Determination

6. While the solution is in the constant-temperature bath, select from Table XIII below the Brookfield spindle-speed combination corresponding to the viscosity type of KLUCEL® HPC being tested. Determine the viscosity of the KLUCEL within 2 hrs after removing it from the stirrer. If the solution stands longer than 2 hrs, return it to the stirrer for 10 min, place it in a bath for 30 min, and then determine viscosity.
7. Remove the bottle from the constant-temperature bath and stir the solution by hand for 10 sec, using a stirring rod. Avoid shaking or vigorous stirring, as this will increase air bubbles, which interfere with viscosity measurement.
8. Immediately insert the appropriate Brookfield viscometer spindle into the solution and start the spindle rotating. Allow it to rotate for 3 min before taking the reading.
9. Stop the instrument and read the dial. Multiply the dial reading by the factor corresponding to the speed and spindle used. The result is the viscosity of the solution in centipoises.

C. Viscosity in Ethanol

The viscosity of ethanol solutions of KLUCEL is determined in the same manner as for aqueous solutions, but with the following modification:

1. To minimize evaporation of the ethanol, the bottle may be capped and mechanically shaken to accomplish solution of the KLUCEL instead of stirring the solution by hand.
2. Repeat steps 5, 6, 7, 8, and 9 as described in the Viscosity in Water procedures.

D. Ash Content

The ash components of KLUCEL are sodium salts. Determination of these is complicated by the presence of certain anticaking agents, which contribute to the total ash. The ash method is referenced in the *National Formulary*, current edition.

Table XIII – Sample Weights of KLUCEL, and Brookfield Spindle and Speed for Viscosity Measurement

Viscosity Type	Concentration, %	Sample Wt, g	Brookfield Settings	
	rpm		Spindle No.	
E 10	25.0	30	2	
L 5	11.0	30	1	
J 5	11.0	60	2	
G 2	5.2	60	2	
M 2	5.2	60	4	
H 1	2.3	30	3	

PRODUCT LISTING SUPPLEMENT

The following list of products, along with their chemical identity and source of supply, may be helpful to the reader who is unfamiliar with some of the products referred to in this booklet.

Read and understand the Material Safety Data Sheets (MSDSs) before using these products.

Product	Chemical Identity	Manufacturer
Antifoam AF	Dimethylpolysiloxane materials	Stewart Hall Chemical
Aqualon® CMC	Sodium carboxymethylcellulose	Hercules Incorporated
Benecel® MC	Methylcellulose	Hercules Incorporated
Butyl Cellosolve	Ethylene glycol monobutyl ether-solvent	Dow Chemical Co.
Carbowax 1000	Polyethylene glycol	Dow Chemical Co.
Cellosolve	Ethylene glycol monoethyl ether-solvent	Dow Chemical Co.
Colloid 581-B	Hydrocarbon-based material containing surfactants, waxes, and metallic soaps	Rhône-Poulenc, Inc.
Defoamer 1512	Silica-type defoamer	Hercules Incorporated
Methyl Cellosolve	Ethylene glycol monomethyl ether	Dow Chemical Co.
M-Pyrol	N-methyl-2-pyrrolidone	GAF Corporation
Myvacet	Acetylated monoglyceride	Eastman Chemical Products, Inc.
Myverol	Monoglyceride	Eastman Chemical Products, Inc.
Natrosol® HEC	Hydroxyethylcellulose	Hercules Incorporated
Nopco KFS	Proprietary blend of hydrocarbon oil, fatty acids, wax, and nonionic surfactants	Henkel Corporation
Nopco NDW	Proprietary blend of hydrocarbon oils, silicone, nonionic surfactants, and metallic soaps	Henkel Corporation
Omadine	Sodium 1-hydroxpyridine-2-thione	Olin Chemicals

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EXHIBIT C

Network Structure of Cellulose Ethers Used in Pharmaceutical Applications during Swelling and at Equilibrium

Saša Baumgartner,[1,2] Julijana Kristl,[1,3] and Nicholas A. Peppas[2]

Received March 19 2002; accepted April 23, 2002

Purpose. The purpose of this work was to investigate the swelling behavior of four cellulose ethers that differ in their type and degree of substitution and to elucidate the network structure of the swollen matrices under dynamic and equilibrium conditions.

Methods. Dynamic vapor sorption was performed to assess the ability of polymer chains and water molecules to interact. Dynamic and equilibrium swelling studies were performed to calculate molecular parameters of swollen polymers using the Flory-Rehner theory.

Results. We determined the volume-swelling ratio of the polymer matrices and observed that it was dependent on their hydrophilicity. We determined molecular parameters that characterize the swollen hydrogels of cellulose derivatives, such as the polymer volume fraction in the swollen state, $v_{2,S}$, the effective molecular weight of the polymer chain between physical entanglements, $\overline{M_e}$, the number of repeating units between two entanglements, u, and the number of entanglements per chain, e. The $\overline{M_e}$ of the cellulose derivatives studied varied significantly depending on the type of cellulose ether and on the swelling time.

Conclusions. The order of mesh size, an important parameter for predicting drug diffusion and release, taking into account all determined parameters, is: hydroxypropyl cellulose < hydroxyethyl cellulose < hydroxypropyl methyl cellulose K100M < hydroxypropyl methyl cellulose K4M.

KEY WORDS: cellulose ethers; swelling; mesh size; network parameters; controlled release.

INTRODUCTION

The chemical and physical characteristics of cellulose derivatives used in pharmaceutical applications have been described in relation to their use in sustained-release formulations (1,2). Upon contact with water, tablets start to swell, forming a gel layer around the dry core. Chain dissolution may take place at the gel surface depending on the type of cellulose ether. Numerous investigators, including our group, have studied these two processes (1,3–6). Significant models have been proposed by Peppas and collaborators (7–9) to describe swelling and erosion of polymeric carriers used in pharmaceutical formulations, as well as the water uptake and drug release behavior.

In general, because the dry core of polymer tablets is glassy, the drug contained in them cannot diffuse unless swelling takes place. On swelling, drug molecules dissolve in water and are released by diffusion. The processes of swelling, erosion, and drug release can occur simultaneously and are interconnected (7–9). Thus, it is rather difficult to develop mathematical models that include all interrelated parameters and that fit accurately the experimental results. Recently, in a series of pioneering contributions, Siepmann *et al.* (10,11) developed numerous accurate models that describe all the processes that take place during the drug release from hydroxypropyl methyl cellulose (HPMC) tablets. Yet, even in these models, there has been no previous analysis to characterize or estimate the molecular structure and size of the continuously swelling matrix during drug release, particularly not for tablets based on cellulose derivatives. To better identify the necessary parameters that will characterize the molecular structure of swollen gels produced by hydration of the cellulose derivatives typically used in pharmaceutical formulations, one needs to examine in more detail the mechanism of swelling of such carriers.

Most hydrophilic cellulose derivatives form hydrogels. To evaluate the feasibility of using a particular hydrogel as a drug-delivery device, it is important to know the structure and properties of the associated polymer network that forms during swelling. As reported earlier (9–11), such networks can form when compressed tablets, e.g., tablets of HPMC, are placed in water or in physiological fluids. Then, individual particles swell and their macromolecular chains start entangling, thus creating diffusional spaces that are controlled by the molecular weight and hydrophilic characteristics of the carrier polymers. Evidently, the average distance between consecutive physical entanglements, tie junctions, or tie points in these physical networks is a most important molecular parameter that will control not only the integrity of the formed swollen network (hydrogel) but also the diffusional characteristics of the drug diffusing through it and being released. This average distance is often called the 'mesh size' and can be expressed either in units of molecular weight (daltons) or in units of length (typically nm).

From a thermodynamic point of view, the most important parameters that define the behavior of these swollen hydrogels are the polymer volume fraction in the swollen state, $v_{2,S}$, the average molecular weight of the polymer chains between crosslinked points, $(\overline{M_c})$, and the associated mesh size, ξ. These parameters can be mutually dependent and are determined either theoretically or experimentally (12,13). Each approach has its own basic theory, with limitations in validity and certain approximations.

The aim of this work was to investigate the swelling of discs of cellulose ethers widely used in pharmaceutical formulations and to quantify the molecular parameters that characterize the diffusional pathway for drug transport. To our knowledge, this is the first time that such an approach has been presented for matrix and gel-forming cellulose derivatives.

MATERIALS AND METHODS

Materials

Cellulose derivatives used were hydroxyethyl cellulose (HEC, Natrosol 250 - HHX, Aqualon, Hercules; $(\overline{M_w}) \approx$

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1,200,000, molar substitution ≈ 2.5), hydroxypropyl cellulose (HPC, Klucel 99 - HXF, Aqualon, Hercules; ≈ 1, 150, 000, molar substitution ≈ 3.7), hydroxypropyl methyl cellulose (HPMC, Premium Methocel K4M, Colorcon; ≈ 95, 000, methoxyl groups = 22.9% and hydroxypropoxyl groups = 9.2), and HPMC (Premium Methocel K100M, Colorcon; ≈ 250,000, methoxyl groups = 22.4% and hydroxypropoxyl groups = 10.4).

Methods

Preparation of Disc-Like Specimens

Discs of the various cellulose derivatives were prepared by compressing 500 mg of powder using round flat punches of 12 mm in diameter (tableting machine EKO; Korsch, Germany). Discs (n = 6) of hardness 100 N ± 10 (VanKel VK 200, Cary, NC, USA) were prepared. Before swelling tests, the diameter and height of each tested disc were measured.

Water Sorption Studies

Water sorption experiments of polymeric powders were conducted in a vapor sorption device (DVS, Surface Measurement Systems Ltd, UK). Gas of known relative humidity was passed over a small sample of the polymer carrier to be tested. The sample was hung on a loop attached to a microbalance that monitors the sample mass as a function of time. The temperature was controlled at 26°C, and the sample mass varied from 1 to 1.5 mg. The relative humidity (%) was varied stepwise from 0 to 95% and back over a period of 30 h.

Preparation of Hydrogels

Each cellulose ether hydrogel sample was prepared in three different weight percent concentrations. Accurately weighed HPMC polymer samples were first dispersed in purified water to form a 90% solution, heated at 80 to 90°C, and stirred with a magnetic stirrer. The remainder of the cold water was added, and the hydrogel cooled to room temperature. HPC hydrogels were prepared in the same way except that the water was heated at 45 to 50°C, whereas HEC hydrogels were prepared at 25°C.

True Density Determination

The true density of polymer discs (ρ_t) was measured using a helium pycnometer (AccuPyc1300, Mycromeritics, Norcross, GA, USA) at 24°C ± 1.

Swelling Studies

Swelling studies were performed by placing the polymeric discs in test tubes and measuring their thickness as a function of time during swelling as shown in Fig. 1. Tubes were kept vertical at 37°C.

RESULTS AND DISCUSSION

Adsorption Isotherms and Hydrophilicity of Cellulose Ethers

Water sorption measurements were used to study the influence of polymer chemical structure on polymer-water

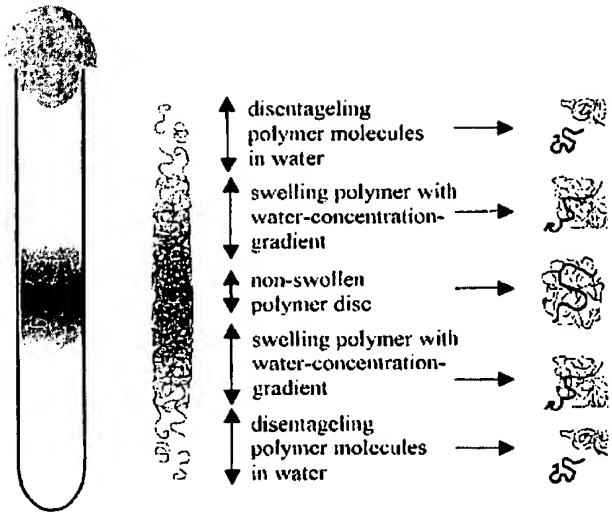

Fig. 1. Experimental set-up of the swelling experiment.

interactions. As shown in Fig. 2, the water adsorption isotherms of HPMC, HPC, and HEC had the same general shape and were characteristic of hydrophilic polymers. HEC was the most hygroscopic polymer, followed by HPMC K4M, HPMC K100M, and HPC. There was no significant hysteresis effect because the polymer mass differences between sorption and desorption were small, i.e., around 2% for HPMC polymers, around 0.7% for HEC polymer, and less than 0.1% for HPC.

Hydroxyethyl substituents of HEC polymer are bound primarily to the hydroxyl group of the basic β-glucopyranose unit and secondarily to the other hydroxyethyl group. There are many possibilities for the interactions between water molecules and unsubstituted hydroxyl and side chain hydroxyethyl groups. HPMC polymers have besides hydrophilic hydroxypropyl substituents also hydrophobic methoxyl groups, and hence exhibited a lower hygroscopicity than HEC. The

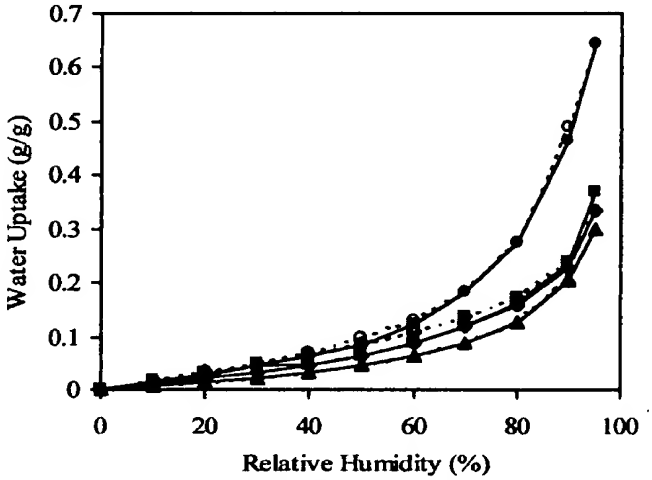

Fig. 2. The change in mass of bulk polymers at equilibrium under different relative humidity using the vapor sorption method: full line, full markers: sorption; dashed line, empty markers: desorption; HEC (●, ○); HPMC K100M (■,□); HPMC K4M(◆,◇); and HPC (▲, Δ).

molar substitution of HPC polymer was 3.7, indicating that most hydroxyl groups are substituted with hydrophilic hydroxypropyl groups, providing many possibilities for interaction between water and the polymer molecules. These interactions are favorable on the surface of HPC. In addition to these interactions, there must be strong interactions between polymer chains, accounting for the low hygroscopicity determined by vapor sorption measurements.

Dynamic and Equilibrium Swelling Studies

The dynamic swelling behavior of hydrogel discs was studied by measuring the thickness of the gel layer formed as swelling occurred in the presence of water at 37°C. Swelling experiments were continued until equilibrium was reached, i.e., until the gel thickness became essentially constant. As noted before by Colombo *et al.* (14), during swelling of the hydrophilic cellulose derivative (HPMC), the macromolecular chains absorb water, leading to an expansion of the network formed and to formation of a quasi-equilibrium structure. This three-dimensional network structure usually is held together by physical chain entanglements, hydrogen bonds, tie junctions, or tie points produced by various types of forces. Upon further absorption of water, these gels may start disentangling, indicating a competitive phenomenon of swelling and dissolution. In our experiments, swelling predominated, as shown by the substantially constant volume of the measured thickness. However, it is important to determine the nature of this three-dimensional network for all cellulose derivatives, even those that might eventually erode.

Figure 3A shows typical plots of the discs thickness as a function of time. The different matrix discs reached equilibrium at different times. The equilibrium state for HEC was reached after 240 h, for HPC and HPMC K100M after 260 h, and for HPMC K4M after 170. The thickness of the HEC gel at equilibrium was almost 4.5 cm. The comparison between different hydrogels is best seen from data normalized to the thickness of the starting discs (Fig. 3B).

The thickness of the samples increased in the following order: HPC, HPMC K4M, HPMC K100M, and HEC up to 190 h of swelling. Beyond that time, differences between HPC and HPMC K4M gel thickness were no longer significant (Fig. 3B). A comparison of swelling data of HPMC polymers showed that equilibrium for HPMC K100M was reached approximately 120 h later than for HPMC K4M. The reason for this phenomenon is that disentanglement of HPMC K100M, and hence erosion, was hindered because of the longer polymer chains. Again, it must be noted that although erosion by disentanglement (7,15) could eventually occur, these studies are relevant for any cellulose material used in tablets.

The thickness of different polymeric discs can be used directly to predict drug release because it is a parameter as relevant to the release process as the swelling front and gel layer parameters used by Colombo *et al.* (8). Of course, in real USP 25, 2001 testing of drug release, swelling, erosion, and release take place in a dissolution apparatus or in a physiological environment where erosion might be somewhat faster. Yet, the overall phenomena observed here are not different. In drug release from polymer matrices under USP conditions, the release is controlled by a combination of swelling, disentanglement and erosion (9,10), and the characteristics of these processes are rate-limiting parameters in drug diffusion (15). Therefore, to use these data for drug release prediction it is necessary to obtain quantitative information about the molecular network structure of the gels.

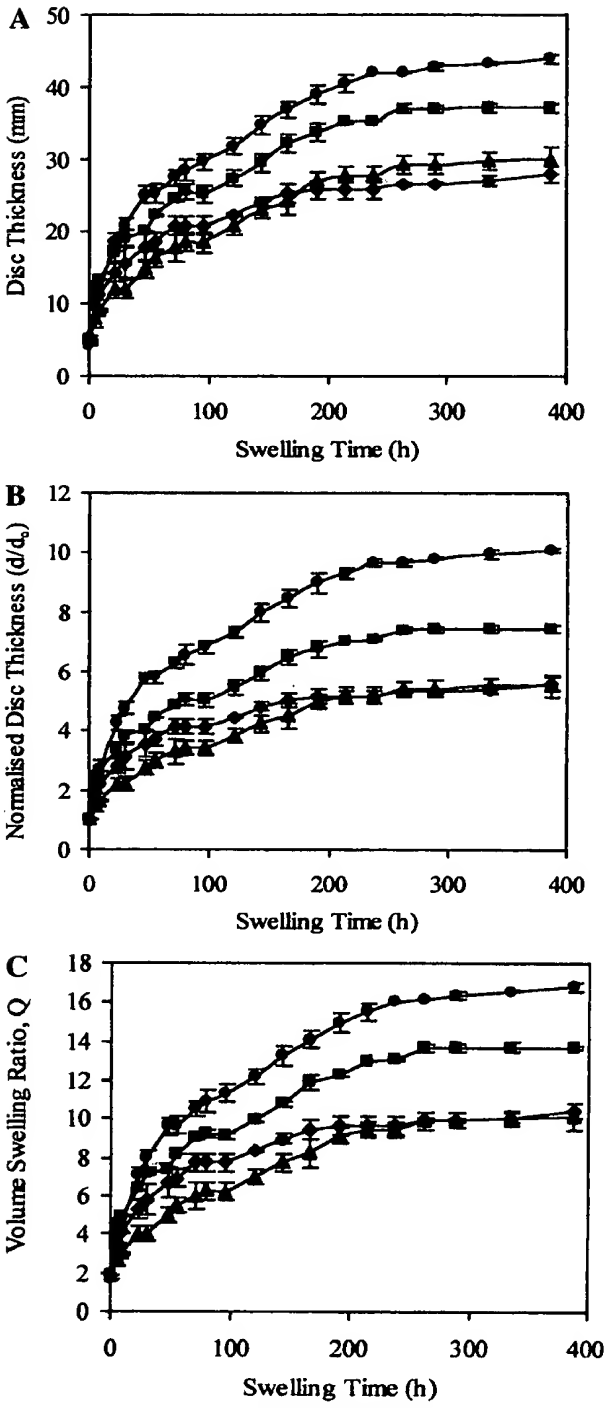

Fig. 3. (A) Thickness of the gel layer formed in a polymer disc as a function of swelling time. (B) Normalized thickness of the gel layer formed in a polymer disc as a function of swelling time. (C) The volume swelling ratio, Q, as a function of swelling time: HEC (●); HPMC K100M (■); HPMC K4M (◆); and HPC (▲).

Volume Swelling Ratio

The dynamic gel thickness data obtained earlier were translated into a volume-swelling ratio, Q, using the following equation:

$$Q = V_s/V_d \quad (1)$$

where V_s is the volume of swollen gel and V_d is the initial volume of the dry disc.

Results of the dynamic swelling behavior of hydrophilic, cellulose-based tablets are presented in Fig. 3C. The volume-swelling ratio correlated well with the hydrophilicity of the polymer determined from vapor sorption measurements. As expected, comparison of the HPMC polymers showed that HPMC K100M, which has a higher molecular weight than HPMC K4M, exhibited a higher Q.

Average Molecular Mass between Physical Entanglements at Equilibrium

The results of gel layer thickness were used to determine the average molecular weight between consecutive entanglements or joints or links by the use of an appropriate theoretical model. In general, the average molecular weight between two consecutive entanglements, ($\overline{M_e}$), can be calculated (12) from the Flory-Rehner equation. This model describes the equilibrium volume-swelling ratio of crosslinked polymers based on the postulate that the elastic retractive forces of the polymer chains and the thermodynamic compatibility of the polymer with the solvent molecules balance each other during swelling. This assumption is valid when the hydrogels are neutral, swelling is isotropic, there are tetra-functional crosslinks at zero volume, four chains are connected at one point, polymer chains are crosslinked in the solid state, and where Gaussian distribution of the polymer chain is assumed (12,13).

The $\overline{M_e}$ was calculated by

$$\frac{1}{\overline{M_e}} = \frac{2}{\overline{M_n}} - \frac{(v/V_1)[\ln(1-v_{2,s}) + v_{2,s} + \chi_1 v_{2,s}^2]}{\left(v_{2,s}^{1/3} - \frac{v_{2,s}}{2}\right)} \quad (2)$$

where $\overline{M_n}$ is the number average molecular weight of the cellulose ether tested, v is its specific volume, V_1 is the molar volume of water, $v_{2,S}$ is the polymer fraction in the swollen gel at equilibrium ($v_{2,S} = 1/Q$), and χ_1 is the Flory or polymer-water interaction parameter (12,13). Values of $\overline{M_n}$ for the cellulose ethers used in these studies are summarized in Table I. Values for HPMC are those provided by the manufacturer, whereas for HEC and HPC they are obtained from $\overline{M}_n$ - $\overline{M}_w/2$. (16). The specific volume of the polymer was the reciprocal value of the true density (Table I), and V_1 was the molar volume of deionized water, 18.1 cm^3/mol.

The polymer-water interaction parameter, χ_1, was introduced independently by Flory and Huggins (12,13). In general, for a polymer to be soluble in water at a particular temperature, χ_1 must be below 0.5. If χ_1 is only slightly greater than 0.5, the polymer will be poorly swollen in water, and the latter is considered as a poor solvent or swelling agent. However, thermodynamically real polymer-water systems do not conform closely to the Flory-Huggins model, and the border value of 0.5 is not strictly valid. Experimental values of χ_1 and their dependence on composition, temperature, and molecular weight provide useful indications of the nature and extent of polymer-water interaction (17). Approximate values of the χ_1 parameter for cellulose ethers in water were used (Table I) on the basis of their chemical structure and interaction with water, determined from sorption measurements.

$\overline{M_e}$ was calculated for each polymer disc under equilibrium swelling conditions (Table I). The resulting order of increasing $\overline{M_e}$ for the cellulose ethers was as follows: HPMC K4M < HPC < HPMC K100M < HEC.

To support the validity of this order, we calculated $\overline{M_e}$ for χ_1 parameters between 0.3 to 0.5 to determine the importance of the thermodynamic compatibility between water and the polymer chains for the calculation of this important molecular parameter. The associated results are presented in Table II. The effect of the Flory interaction parameters on values of $\overline{M_e}$ is somewhat important. However, if we compare the values of $\overline{M_e}$ at the same value of the χ_1 parameter for the polymers investigated, their order always remains as HPMC K4M < HPC < HPMC K100M < HEC. It was concluded that, based on experimental data, this order was valid.

In addition, to confirm the validity of determining $v_{2,S}$ using a cathetometer, a method based on the buoyancy principle was also used (12). In a typical experiment, a disc was allowed to swell in water, and at different time intervals its

Table I. The Characteristic Data of Cellulose Ethers Needed for Calculation of $v_{2,s}$ and $\overline{M_e}$ of Hydrogels Formed on Discs in an Equilibrium Swelling Experiment

Polymer disc	Hydroxyethyl cellulose	Hydroxypropyl cellulose	Hydroxypropyl methyl cellulose K4M	Hydroxypropyl methyl cellulose K100M
True density (g/cm^3)	1.3603	1.1985	1.3196	1.3145
$\overline{M_n}^a$	600,000	570,000	9,000	125,000
v^b	0.73519	0.83626	0.75781	0.76075
χ_1^c	0.35	0.45	0.40	0.40
$v_{2,s}^d$	0.05956	0.09897	0.09600	0.07262
$\overline{M_e}^e$	13,996	10,563	2,868	11,236

[a] Number average molecular weight of polymer.
[b] Specific volume of the polymer (cm^3/g).
[c] Flory interaction parameter or polymer liquid interaction parameter.
[d] Polymer fraction in the swollen gel.
[e] Average molecular weight between two consecutive entanglements.

Table II. Calculated Values of $\overline{M}_e$ (Eq. 2) for Cellulose Ethers at Different Values of χ_1

χ_1 Parameter	$\overline{M}_e$ Hydroxyethyl cellulose	$\overline{M}_e$ Hydroxypropyl cellulose	$\overline{M}_e$ Hydroxypropyl methyl cellulose K4M	$\overline{M}_e$ Hydroxypropyl methyl cellulose K100M
0.30	10,942	3,882	2,259	6,797
0.35	13,996	4,949	2,528	8,470
0.40	19,415	6,712	2,868	11,236
0.45	31,680	10,563	3,314	16,682
0.50	86,038	24,881	3,926	32,377

mass was measured in air and in a non-solvent (heptane). The value of $\upsilon_{2,S}$ could thus be calculated leading to $\overline{M}_e$. Unfortunately, this technique was somewhat difficult to apply to cellulose ether discs.

Therefore, cellulose ether hydrogels were prepared at different polymer concentrations. A hydrogel sample was first weighed in air, w_a, then in heptane, w_h, and, knowing the density of heptane, ρ_h, the volume of swollen gel, V_s, was calculated from

$$V_s = \frac{w_a - w_h}{\rho_h}. \tag{3}$$

The volume of polymer, V_p, in a swollen hydrogel sample was determined from

$$V_p = \frac{w_a \cdot (c/100)}{\rho_p}, \tag{4}$$

where c is the weight percent of polymer in hydrogel sample, and ρ_p is the density of the dry polymer.

Finally $\upsilon_{2,s}$ was calculated from Eq. (5) and $\overline{M}_e$ using Eq. (2)

$$\upsilon_{2,s} = \frac{V_p}{V_s}. \tag{5}$$

The relationship between $\overline{M}_e$ and polymer concentration (5 to 25 w/w%) in the hydrogels was established by fitting the experimental $\overline{M}_e$ values to an exponential function. The fits and phenomenological equations are presented in Fig. 4. As expected, the higher the concentration of the gel samples, the higher the $\upsilon_{2,S}$ and the lower the $\overline{M}_e$ values.

Additionally, the values of $\overline{M}_e$ for swollen discs in equilibrium (Table I) were placed into the phenomenological equations (Fig. 4) and the corresponding concentrations calculated. The obtained weight percent of polymers in swollen discs at equilibrium were 11.2% for HPMC K4M, 9.3% for HPMC K100M, 11.4% for HPC, and 9.8% for HEC. To check the validity of calculated values, the swollen discs were dried to constant weight at the end of the swelling experiment. Concentrations of polymers in the swollen discs thus determined were: 8.6% for HPMC K4M, 9.5% for HPMC K100M, 12.3% for HPC, and 8.4% for HEC discs. These concentrations were in close agreement with those determined based on the predicted model $\overline{M}_e = f(c)$. The methods for determining $\upsilon_{2,S}$ using a cathetometer or by weighing samples are thus comparable.

Calculation of Other Structural Parameters of the Network

Based on the results in Table I and the order of increasing $\overline{M}_e$ for different cellulose ethers, it may be expected that the mesh size of these hydrogels under equilibrium conditions would follow the same order. The average molecular weight between entanglements, which determines the mesh size, could be used to calculate the number of repeating units between two consecutive entanglements, u, using Eq. (6), where U is the molecular weight of each polymer repeating unit.

$$\frac{\overline{M}_e}{U} = u \tag{6}$$

The approximate molecular weights of the repeating units of each polymer were calculated as shown in Table III. The calculated values of u are direct estimates of the mesh sizes in hydrogels, if we assume that the size (in nm) between two consecutive anhydroglucose units, substituted or not, is the same. At equilibrium, the mesh size of HPMC K100M, estimated from u, was the largest, followed by HEC, HPC, and HPMC K4M. However, the mesh size in the hydrogel is probably somewhat smaller than that predicted from the calculation using u, especially for HPC, where the hydroxypropyl substituent is large and the molar substitution is high.

It was also possible to calculate the number of junctions or entanglements, e, per original chain using Equation (7):

$$\frac{\overline{M}_n}{\overline{M}_e} - 1 = e \tag{7}$$

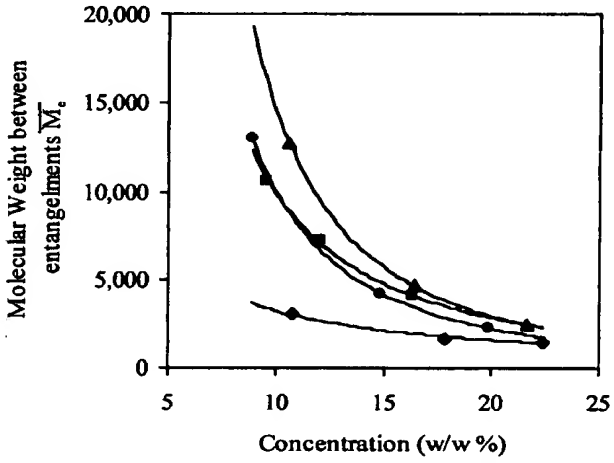

Fig. 4. The relationship between average molecular weight between entanglements, $\overline{M}_e$, and concentration of different cellulose ether in hydrogels: HEC (●); HPMC K100M (■); HPMC K4M (◆); and HPC (▲). The phenomenological equations that describe the fitted curves, are: HEC: $\overline{M}_e = 2 \cdot 10^6 \cdot (concentration)^{-2.179}$; HPMC K100M: $\overline{M}_e = 630{,}000 \cdot (concentration)^{-1.8046}$; HPMC K4M: $\overline{M}_e = 35{,}000 \cdot (concentration)^{-1.042}$; and HPC: $\overline{M}_e = 3 \cdot 10^6 \cdot (concentration)^{-2.3241}$.

Table III. The Molecular Weight of Each Polymer Repeating Unit, U, the Number of Repeating Units between Two Entanglements, u, and the Number of the Entanglements per Original chain, e, at Equilibrium

Polymer	Hydroxyethyl cellulose	Hydroxypropyl cellulose	Hydroxypropyl methyl cellulose K4M	Hydroxypropyl methyl cellulose K100M
U	272	376	187	189
u	51	28	15	60
e	42	53	2	10

where e is calculated for the equilibrium state (Table III). The results of this analysis require further explanation. For example, a HEC disc forms a very thick hydrogel layer in the swollen state. The value of $v_{2,S}$ is the lowest, signifying that the largest amount of water is physically and chemically trapped in the HEC hydrogel structure. As a logical consequence, the mesh size and water content are high compared to those of the other polymers investigated. Because of a high degree of polymerization and high $\overline{M}_n$, the number of entanglements, e, is relatively high, which holds the network structure together at equilibrium.

HPC discs form the thinnest hydrogel layers and the $v_{2,S}$ is relatively high (Table I). This means that the interactions between the HPC chains are very strong and that water molecules occupy a smaller volume than in the case of HEC. Estimation of the mesh size based on $\overline{M}_e$ results would lead to an incorrect conclusion. Indeed, the mesh size of HPC would be expected to be just 1.3 times smaller than that for HEC. However, based on u numbers, the mesh size of HPC is 1.8 times smaller than for HEC and the number of entanglements is higher for 11 (Table III). In addition, the molar substitution for HPC is relatively high and the hydroxypropyl substituents occupy a large volume, which means that the mesh size is probably more than 1.8 times smaller than for HEC. The network structure of HPC hydrogel is significantly different from that of the HEC hydrogel despite almost the same average molecular weight of these polymers.

The thickness of the HPMC K100M gel layer is a little smaller than HEC gel layer (Fig. 3A). The value of $v_{2,S}$ for HPMC K100M is higher than for HEC and lower than for HPC. The mesh size predicted from $\overline{M}_e$ is also intermediate between HEC and HPC but, from the numbers of u, HPMC K100M hydrogel has the largest mesh size of all the polymers, and the fewest entanglements. This can also be the consequence of a lower $\overline{M}_n$ than for HEC and HPC. Compared with HPMC K4M, where the number of entanglements per chain is just 2, which could be described as almost a colloid dispersion or very weak gel, HPMC K100M is much more entangled. The mesh size for HPMC K4M predicted from the u number is surprising, being the smallest of all those investigated. However, the thickness of the gel layer for HPMC K4M can be compared to the gel layer of HPC. However, HPC is much more entangled than HPMC K4M. In addition, the molar substitution is higher.

Therefore, to predict drug release from these cellulose ethers, more realistic data obtained from dynamic swelling are required.

Changes of Molecular Parameters during Swelling

From the alteration of $\overline{M}_e$ with time shown in Fig. 5A, it can be seen that the rate of approach to the equilibrium $\overline{M}_e$ value is the highest for HEC, followed by HPMC K100M, HPC and HPMC K4M. As mentioned previously, $\overline{M}_e$ is proportional to the mesh size indicating that the release of incorporated drug in those matrices would be expected to be of the same order. However, the order of HPMC K100M and HPMC K4M would be reversed because release from HPMC K4M would be faster because of the fact that the number of entanglements is lower than in HPMC K100M hydrogel (see

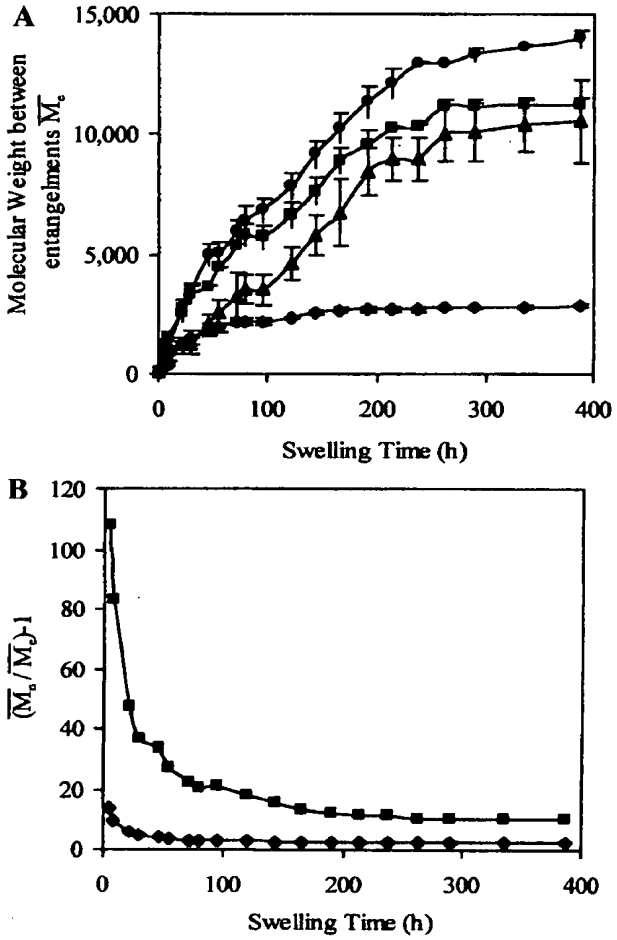

Fig. 5. Changes of molecular parameters during swelling. (A) The calculated average molecular weight between entanglements, $\overline{M}_e$, as a function of swelling time: HEC (●); HPMC K100M (■); HPMC K4M (◆); and HPC (▲). (B) The number of entanglements, e, per chain as a function of swelling time for HPMC discs of different polymer molecular weights: HPMC K100M (■) and HPMC K4M (◆).

also Fig. 5B). Consequently, the process of erosion of HPMC K4M tablets would be faster, as observed also by other authors (4).

CONCLUSIONS

From the present study it can be concluded that the results of dynamic vapor sorption measurements for cellulose ethers correlated well with the volume-swelling ratio. The $\overline{M_c}$, was calculated from the following parameters: the polymer fraction in the swollen gel at equilibrium, $v_{2,S}$, the number average molecular weight of the polymer, $\overline{M_n}$, its specific volume, v, the molar volume of the solvent, V_1, and the χ_1 parameter. The order of $\overline{M_c}$ values for the four cellulose ethers is HPMC K4M < HPC < HPMC K100M < HEC. The number of repeating units between two entanglements, u, and the number of entanglements per chain, e, which describe the mesh size, were also obtained. Comparison of the mesh sizes at equilibrium for the different cellulose ethers showed that HPMC K100M were the biggest, followed by HEC, HPC, and HPMC K4M.

To predict the behavior of drug release, however, the number of entanglements per chain, e, also has to be taken into account, and this parameter has been shown to be lowest for HPMC K4M, followed by HPMC K100M, HEC, and HPC, which is the most entangled of those investigated.

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REFERENCES

1. C. D. Melia. Hydrophilic matrix sustained release systems based on polysaccharide carriers. *Crit. Revs. Ther. Drug Carrier Systems* **8**:395–421 (1991).
2. E. Doelker. Cellulose derivatives. *Adv. Polym. Sci.* **107**:200–265 (1993).
3. P. Gao and R. H. Meury. Swelling of hydroxypropyl methylcellulose matrix tablets. 1. Characterisation of swelling using a novel optical imaging method. *J. Pharm. Sci.* **85**:725–731 (1996).
4. P. Gao, J. W. Skoug, P. R. Nixon, T. R. Ju, N. L. Stemm, and K. C. Sung. Swelling of hydroxypropyl methylcellulose matrix tablets. 2. Mechanistic study of the influence of formulation variables on matrix performance and drug release. *J. Pharm. Sci.* **85**:732–740 (1996).
5. S. Baumgartner, J. Šmid-Korbar, F. Vrečer, and J. Kristl. Physical and technological parameters influencing floating properties of matrix tablets based on cellulose ethers. *S.T.P. Pharma. Sci.* **8**: 285–290 (1998).
6. K. Tahara, K. Yamamoto, and T. Nishihata. Overall mechanism behind matrix sustained release (SR) tablets prepared with hydroxypropyl methylcellulose 2910. *J. Control. Release* **35**:59–66 (1995).
7. B. Narasimhan and N. A. Peppas. Molecular analysis of drug delivery systems controlled by dissolution of the polymer carrier. *J. Pharm. Sci.* **86**:297–304 (1997).
8. P. Colombo, R. Bettini, and N. A. Peppas. Observation of swelling process and diffusion front position during swelling in hydroxypropylmethyl cellulose (HPMC) matrices containing a soluble drug. *J. Control. Release* **61**:83–91 (1999).
9. J. Siepmann, K. Podual, M. Sriwongjanya, N. A. Peppas, and R. Bodmeier. A new model describing the swelling and drug release kinetics from hydroxypropyl methylcellulose tablets. *J. Pharm. Sci.* **88**:65–72 (1999).
10. J. Siepmann, H. Kranz, R. Bodmeier, and N. A. Peppas. HPMC-Matrices for controlled drug delivery: A new model combining diffusion, swelling, and dissolution mechanisms and predicting the release kinetics. *Pharm. Res.* **16**:1748–1756 (1999).
11. J. Siepmann and N. A. Peppas. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Adv. Drug Deliv. Revs.* **48**:139–157 (2001).
12. N. A. Peppas and B. D. Barr-Howell. Characterization of the cross-linked structures of hydrogels. In N. A. Peppas (ed.), *CRC Hydrogels in Medicine and Pharmacy*, vol 1, Fundamentals, CRC Press, Boca Raton, FL, 1986, pp. 27–57.
13. A. M. Lowman and N. A. Peppas. Hydrogels. In: E. Mathiowitz (ed.), *Encyclopedia of Controlled Drug Delivery*, Wiley, New York, 2000, pp. 397–417.
14. P. Colombo, R. Bettini, and N. A. Peppas. Swelling matrices for controlled drug delivery: Gel layer behavior, mechanisms and optimal performance. *Pharm. Sci. Technol. Today* **3**:198–204 (2000).
15. J. Siepmann and N. A. Peppas. Hydrophilic matrices for controlled drug delivery: An improved mathematical model to predict the resulting drug release kinetics (the "Sequential Layer" model). *Pharm. Res* **17**:1290–1298 (2000).
16. M. G. Wirick. Study of the substitution pattern of hydroxyethylcellulose and its relationship to enzymic degradation. *J. Polymer Sci.* **6**:1705–1718 (1968).
17. A. F. M. Barton. *CRC Handbook of Polymer-Liquid Interaction Parameters and Solubility Parameters*, CRC Press, Boca Raton, FL, 1990, pp. 3–22.

EXHIBIT D

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Rheological Characterization of Neutral and Anionic Polysaccharides With Reduced Mucociliary Transport Rates

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ABSTRACT

The purpose of this research was to compare the viscoelastic properties of several neutral and anionic polysaccharide polymers with their mucociliary transport rates (MTR) across explants of ciliated bovine tracheal tissue to identify rheologic parameters capable of predicting the extent of reduction in mucociliary transport. The viscoelastic properties of the polymer gels and gels mixed with mucus were quantified using controlled stress rheometry. In general, the anionic polysaccharides were more efficient at decreasing the mucociliary transport rate than were the neutral polymers, and a concentration threshold, where no further decreases in mucociliary transport occurred with increasing polymer concentration, was observed for several of the neutral polysaccharides. No single rheologic parameter (η, G′, G″, tan δ, G*) was a good predictor of the extent of mucociliary transport reduction, but a combination of the apparent viscosity (η), tangent to the phase angle (tan δ), and complex modulus (G*) was found to be useful in the identification of formulations capable of decreasing MTR. The relative values of each of the rheologic parameters were unique for each polymer, yet once the relationships between the rheologic parameters and mucociliary transport rate reduction were determined, formulations capable of resisting mucociliary clearance could be rapidly optimized.

KEYWORDS: Mucociliary clearance, rheology, carboxymethylcellulose, hydroxypropyl methylcellulose, methylcellulose, xanthan, alginate.

INTRODUCTION

Efficient mucociliary clearance depends on the mucus layer having particular rheological properties. The viscoelasticity of the mucus layer contributes to the effectiveness of mucociliary clearance, but the interaction between the mucus and the cilia also plays a critical role. Previous investigators have studied the relationship between a material's viscosity and/or elasticity and the resulting mucociliary transport rate.[1-3] Work performed by King et al showed that while there was no specific chemical requirement for transport, all of the systems found to be transported across a mucus-depleted frog palate possessed a slight degree of crosslinking, suggesting an important role for the elastic character of the system.[4] Shih et al showed, using reconstituted lyophilized canine tracheal mucus, that mucociliary clearance increased with increasing elasticity up to an elastic modulus value (G′) of 1 Pa (determined at a frequency of 16 Hz) but then decreased again with further increases in elasticity above this value.[3] Majima et al[5] demonstrated that the maximum clearance rate of mucus on a mucus-depleted frog palate was achieved when the value of G′ was 2 Pa at a frequency of 1 Hz at 25°C. Gelman and Meyer[6] were able to alter the elastic modulus of cervical mucus gels without significantly altering the viscous modulus by crosslinking the mucins with gluteraldehyde; the transport of these cervical mucus samples across the mucus-depleted frog palate correlated with the changes in elastic modulus and showed an optimum value for transport at 0.16 Pa. Chen and Dulfano[2] showed that the most rapid transport across a mucus-depleted frog palate was achieved by sputum with Newtonian viscosity values between 1000 and 3000 Poise determined at shear stresses less than 10 Pa, and Puchelle et al[7] reported the optimum transport rate for xanthan gum across a mucus-depleted frog palate was observed at a viscosity value of 120 Poise determined at a shear rate of 0.4 sec^{-1}. These results indicate that biorheological requirements do exist for optimal mucociliary clearance, yet the variety of methods and materials selected for testing makes it difficult to establish parameter values that can be used to a priori optimize mucociliary transport of drug formulations containing various polymers and added excipients. Other investigators have attempted to correlate rheologic properties of gels and gel-mucus combinations to the mucoadhesive character of the gel.[8-10] Unfortunately, the rheological measurements, while excellent for identifying gel-mucus interactions, were not able to accurately predict mucoadhesion as measured using tensile strength testing.[10]

Identifying the viscoelastic properties of formulations capable of transiently increasing the residence time in the

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respiratory tract while efficiently releasing the drug from the matrix may enable the formulation of bioadhesive systems with improved therapeutic efficacy and minimal toxicity. The objective of these studies was to systematically compare the rheological parameters of a series of chemically related, neutral, and anionic polysaccharide polymers to the reduction in their mucociliary transport across bovine tracheal explants. A knowledge of the parameter ranges that result in the greatest inhibition of mucociliary clearance should greatly improve the ability to rapidly optimize formulations with prolonged mucosal contact time on ciliated mucosal surfaces using in vitro methods.

MATERIALS AND METHODS

Materials

Sodium chloride, potassium chloride, calcium chloride, sodium bicarbonate, dextrose, sodium hydroxide, potassium phosphate (dibasic), potassium phosphate (monobasic), porcine gastric mucin (Type II), sodium azide, activated charcoal, and dithiothreitol (DTT) were obtained from Sigma Chemical Co (St Louis, MO). Methocel A4C and A15C (methylcellulose [MC], molecular weight [MW] 41 000 and 63 000, respectively) and Methocel E4M (hydroxypropyl methylcellulose [HPMC], MW 86 000) were gifts from Dow Chemical Co (Midland, MI). Aqualon 7MF (sodium carboxymethylcellulose [CMC], MW 250 000) was a gift from Hercules, Inc (Wilmington, DE). Vanzan NF (xanthan gum [XAN], MW > 10^6) was obtained as a gift from R.T. Vanderbilt Co, Inc (Norwalk, CT). Dextran (DEX, MW ~500 000) was purchased from Sigma Chemical Co (St Louis, MO). Manugel GHB (sodium alginate [ALG], MW ~10^5) was a gift from International Specialty Products (Wayne, NJ).

Methods

Preparation of Polymer Gels

Both Methocel A4C and A15C (MC) gels were prepared in 1.25%, 2.5%, and 5% wt/vol concentrations, while Methocel E4M (HPMC) gels were prepared in 1.25% and 2.5% wt/vol concentrations. These concentrations were based on previous reports regarding the bioadhesion of these polymers and qualitative estimates of a useful range of viscosities appropriate for intranasal administration.[11-13] Approximately one third of the volume of water required for preparation of each gel was heated to ~90°C, and the polymer was added to the water with agitation by a lab stirrer. The remaining volume was added as cold water or ice. Agitation was continued for at least 30 minutes.

Other polymers investigated included 1%, 2.5%, and 3% wt/vol sodium CMC; 0.125%, 0.45%, and 0.5% wt/vol XAN; 3.5% and 6% wt/vol ALG; and 0.25% and 2.5% wt/vol DEX. These gel formulations were prepared by slowly sifting the polymer into the vortex of room temperature water stirring in a beaker. Mixing was continued for 30 minutes following addition of the polymer.

All of the polymer formulations were allowed to hydrate overnight at room temperature. They were centrifuged at 3000 rpm for 3 minutes (Marathon 21K, Fisher Scientific, Hampton, NH) to remove entrapped air, and the formulations were allowed to rest at room temperature for another 12 hours before any rheological measurements were conducted.

Reconstitution and Purification of Mucus

A reconstituted porcine gastric mucus solution was prepared using a modification of the method reported by List et al.[14] The use of lyophilized porcine gastric mucin was preferred in these studies because of the need for a matrix that contained limited nonglycoprotein contaminants that could contribute to the variability in the rheological properties of the final mucus gel. Lyophilized porcine gastric mucin Type II (40 mg/mL) was suspended in isotonic phosphate buffer (pH 6.6, the pH of mucus at the apical cell surface[15]) containing 0.02% wt/vol sodium azide and stirred overnight at 4°C. Sodium azide prevents the growth of mold and bacteria in the prepared mucus, and its presence has no significant effect on mucociliary clearance in the bovine explants. The resulting suspension was centrifuged at 16 000 rpm for 15 minutes using a refrigerated super-speed Sorvall RC26 Plus centrifuge (Kendro Laboratory Products, Newtown, CT). The supernatant was decanted and centrifuged once again under the same conditions. The final supernatant was placed into cellulose acetate dialysis tubing (MW cutoff 12 000-14 000; Spectrum Chemical Co, Houston, TX) and dialyzed for 24 hours against isotonic phosphate buffer (pH 6.6) at 4°C. The resulting mucus solution (3%-3.5% wt/vol) was stored at 4°C for 2 days before long-term storage at –70°C prior to use.

Preparation of Polymer-Mucus Mixed Gels

Since a polymer-containing formulation will interact with the mucus layer prior to any interaction with the mucosal surface, the rheological characterization of polymers and polymer/mucus systems was performed to gain a better understanding of the role that the viscoelastic properties of polymers and mucus play in mucoadhesion.[8] Polymer-mucus ratios that were reflective of in vivo conditions were prepared by gently mixing 5 parts (by weight) of the polymer gel with 1 part of mucus.[16] Control gels were prepared by diluting the polymer gel with water in a 5:1 ratio. The concentration of the polymers reported for the polymer-mucus

Table 1. Viscoelastic Parameters (at 3.16 Hz) of Neutral Polysaccharide Polymer and Polymer-Mucus Mixtures[†]

Sample	$\eta^{\ddagger}$ (*mPa s*)	G′ (Pa)	G″ (Pa)	Tan δ	G* (Pa)
A4C 1.25%	36 (1.5)	8.9 (2.2)	2.6 (0.4)	0.30 (0.09)	9.3 (2.1)
A4C 1.25%/mucus	44[§] (1.9)	7.7 (1.7)	1.6 (0.6)	0.21 (0.03)	7.8 (1.8)
A4C 2.5%	278 (17)	19 (3.0)	9.5 (0.7)	0.51 (0.10)	21 (2.6)
A4C 2.5%/mucus	309 (11)	27[§] (3.0)	12 (2.2)	0.43 (0.03)	29[§] (3.6)
A4C 5%	2722 (335)	84 (15)	88 (9.2)	1.1 (0.09)	121 (17)
A4C 5%/mucus	2936 (143)	127[§] (14)	101 (10.7)	0.79[§] (0.04)	162[§] (17)
A15C 1.25%	58 (3.4)	7.2 (1.6)	2.3 (0.4)	0.33 (0.09)	7.6 (1.5)
A15C 1.25%/mucus	74[‡] (4.5)	9.5 (1.6)	2.8 (0.6)	0.30 (0.01)	10 (1.6)
A15C 2.5%	515 (7.0)	40 (7.4)	19 (2.5)	0.48 (0.10)	44 (7.0)
A15C 2.5%/mucus	535 (44)	22[§] (6.0)	17 (3.1)	0.80[§] (0.11)	28[§] (6.4)
A15C 5%	3157 (155)	100 (11)	105 (25)	1.0 (0.16)	145 (25)
A15C 5%/mucus	2817 (258)	99 (6.4)	93 (4.5)	0.94 (0.02)	136 (7.7)
HPMC 1.25%	133 (7.7)	3.4 (0.9)	3.8 (0.3)	1.2 (0.22)	5.1 (0.8)
HPMC 1.25%/mucus	128 (8.5)	4.9 (0.6)	4.4 (0.7)	0.91 (0.15)	6.6 (0.7)
HPMC 2.5%	928 (142)	23 (5.5)	28 (3.1)	1.3 (0.18)	36 (5.8)
HPMC 2.5%/mucus	718 (68)	30 (4.6)	33 (1.7)	1.1 (0.12)	45 (4.2)
DEX 0.5%	2.3 (0.1)	ND	ND	ND	ND
DEX 0.5%/mucus	2.5 (0.7)	ND	ND	ND	ND
DEX 2.5%	2.9[‡] (0.01)	ND	ND	ND	ND
DEX 2.5%/mucus	3.7 (0.1)	ND	ND	ND	ND

[†]All values are means of 3 replicate determinations; values in parentheses are SDs. A4C indicates methylcellulose [MW 41000]; A15C, methylcellulose [MW 63000]; HPMC, hydroxypropyl methylcellulose; DEX, dextran; ND, not detectable.
[‡]Values for polymer-mucus mixtures significantly different than polymer ($P<0.05$).
[§]Apparent viscosity obtained from constant rate flow curve at 100 s^{-1}.

mixtures (Table 1 and Table 2) indicates the concentration of the polymer before mixing with mucus.

Rheological Measurements

The rheological properties of the polymer formulations were determined with a Haake RS1 controlled stress rheometer using a cone and plate sensor system (C60/4, 60 mm diameter, 4° angle) connected to a Haake F3-CH temperature control system equipped with V2.97 data acquisition software (Haake Mess-Technik GmbH Co, Karlsruhe, Germany). The measurement gap distance was fixed at 0.138 mm. All tests were run at 35°C to simulate the temperature of the nasal mucosal surface.[17] To minimize dehydration of the sample during rheologic testing, a solvent trap was used to cover the sample during analysis.

Stress amplitude sweep tests (0.1-40 Pa) at a fixed frequency of 3.16 Hz were conducted to determine the complex modulus as a function of applied stress. This frequency was selected to mimic the reported in vivo ciliary beat frequency.[18] A stress value (0.1 Pa), selected from the linear viscoelastic region, was used for frequency sweep testing where the oscillatory frequency was increased from 0.05 to 5 Hz. The rheological parameters measured during the oscillatory testing included the elastic modulus (G′), viscous modulus (G″), complex modulus (G*), and tan δ (G″/G′). The apparent viscosity of the sample was measured after applying a constant shear rate of 100 sec^{-1} for a period of 1 minute. The value reported for the apparent viscosity was the average of the values obtained during the final 30-second interval of the measurement period.

Measurement of Mucociliary Transport Rate

A modified in vitro technique using bovine tracheal tissues was used to measure the reduction in mucociliary clearance induced by the gels.[2] Tracheal tissues were obtained from local abattoirs and maintained in Locke-Ringer's solution (LR) at room temperature during transport to the laboratory.

Table 2. Viscoelastic Parameters (at 3.16 Hz) of Anionic Polysaccharide Polymers and Polymer-Mucus Mixtures[†]

Sample	η[‡] (mPa s)	G′ (Pa)	G″ (Pa)	Tan δ	G* (Pa)
CMC 1%	23 (0.5)	ND	ND	ND	ND
CMC 1%/mucus	20 (0.03)	ND	ND	ND	ND
CMC 2.5%	278 (22)	3.6 (0.7)	6.9 (0.2)	1.9 (0.4)	7.8 (0.4)
CMC 2.5%/mucus	299 (14)	4.2 (0.1)	7.4 (0.7)	1.8 (0.2)	8.5 (0.6)
CMC 3%	493 (9.3)	7.3 (0.5)	12 (0.6)	1.6 (0.05)	14 (0.7)
CMC 3%/mucus	556 (54)	8.2 (0.5)	13 (0.8)	1.6 (0.02)	15 (0.9)
XAN 0.125%	15 (0.2)	ND	ND	ND	ND
XAN 0.125%/mucus	9.7 (0.2)	ND	ND	ND	ND
XAN 0.45%	67 (1.2)	5.4 (0.1)	2.3 (0.2)	0.43 (0.02)	5.9 (0.2)
XAN 0.45%/mucus	58 (1.8)	6.1[§] (0.3)	2.4 (0.2)	0.40 (0.02)	6.6[§] (0.3)
XAN 0.5%	72 (4.5)	6.3 (0.4)	2.7 (0.2)	0.43 (0.02)	6.9 (0.5)
XAN 0.5%/mucus	73 (2.0)	7.2 (0.4)	2.7 (0.1)	0.38 (0.02)	7.7 (0.4)
ALG 3.5%	513 (17)	1.5 (0.5)	12 (1.2)	8.6 (3.4)	12 (1.1)
ALG 3.5%/mucus	439 (42)	2.4 (0.8)	13 (3.3)	5.4 (1.0)	13 (3.4)
ALG 4%	678 (18)	2.3 (0.1)	16 (0.6)	7.2 (0.5)	16 (0.6)
ALG 4%/mucus	714 (1.2)	3.3 (0.5)	19 (2.1)	5.8 (0.3)	20 (2.2)

[†]All values are means of 3 replicate determinations; values in parentheses are SDs. CMC indicates sodium carboxymethylcellulose; XAN, xanthan gum; ALG, sodium alginate; ND, not detectable.
[‡]Apparent viscosity obtained from constant rate flow curve at 100 s^{-1}.
[§]Values for polymer-mucus mixtures significantly different from those for polymer gel ($P < .05$).

Tracheal segments (~8 × 3 cm) were prepared, and the explants were depleted of endogenous mucus by immersion in 0.2 M DTT for 5 minutes prior to the use of the explants for mucociliary transport rate (MTR) measurement.[4] The explants were washed with LR for ~10 minutes and stored at 4°C for 30 minutes. Prior to measurement, each explant was placed within a closed chamber on a gauze pad saturated with LR and warmed to an epithelial temperature of 35°C (~5 minutes). Immediately prior to the conduct of a transport experiment, the explant was quickly immersed in reconstituted porcine gastric mucus solution and then placed back into the chamber for a final 5-minute equilibration period.

The rate of each gel's movement across the tracheal explant was measured by following the movement of ~10 μL of gel placed in the center of the explant. The gels were spiked with charcoal particles (~10 mg/mL) to assist with visualization. Prior to each gel transport measurement, the tracheal explant was calibrated with a control, charcoal-containing mucus suspension placed on the explant in the same manner as the gels.[12] The movement of the charcoal particles entrapped within the gel or mucus was followed using a Stereomaster stereomicroscope (Fisher Scientific, Hanover Park, IL) at a 10× magnification with a 1-cm calibrated eyepiece. The cilia swiftly and cleanly carry the control mucus along the explant at a rate of ~0.6 to 1 cm/min. The transport rate for each polymer gel was reported as the percentage decrease in MTR in the presence of gel compared with the mucus suspension control (Equation 1):

$$\%\ \text{MTR decrease} = \left(\frac{\text{control MTR} - \text{gel MTR}}{\text{control MTR}}\right) \times 100 \qquad (1)$$

After each control and sample pair, the surface of the explant was rinsed with LR to remove any sample or mucus from the previous measurement, and the surface was replenished with reconstituted mucus. Whenever the control clearance rate of the charcoal suspension was observed to be less than 25% of the initial control clearance rate, the explant was discarded and a new explant was conditioned for use. All mucociliary clearance values reported are the mean of 3 replicate determinations (Table 3).

Results and Discussion

Rheological Measurement of Polymer-Mucus Mixtures

The viscoelastic moduli of the polymer-mucus mixtures exhibited qualitatively similar rheological profiles to those of the pure polymer gels. Methocel A4C 2.5%, A4C 5%,

Table 3. Reduction in Mucociliary Transit Rate Across Bovine Tracheal Explants in the Presence of Polysaccharide Gels*

	Mucociliary Transit Rate (cm/min)		
Sample	Control	Sample	% Reduction
A4C 1.25%	0.75 (0.00)	0.41 (0.02)	35 (12)
A4C 2.5%	0.67 (0.09)	0.19 (0.03)	72 (3.0)
A4C 5%	0.72 (0.05)	0.06 (0.05)	91 (7.9)
A15C 0.625%	0.71 (0.10)	0.61 (0.10)	15 (4.1)
A15C 1.25%	0.67 (0.10)	0.18 (0.06)	74 (7.1)
A15C 2.5%	0.61 (0.10)	0.10 (0.06)	87 (6.8)
A15C 5%	0.75 (0.00)	0.10 (0.05)	87 (6.3)
HPMC 1.25%	0.52 (0.03)	0.15 (0.01)	70 (2.8)
HPMC 2.5%	0.60 (0.00)	0.07 (0.01)	88 (0.98)
DEX 0.5%	0.87 (0.13)	0.76 (0.10)	13 (1.6)
CMC 1%	0.90 (0.08)	0.44 (0.05)	51 (1.9)
CMC 2.5%	0.56 (0.03)	0.07 (0.01)	88 (1.2)
CMC 3%	0.56 (0.03)	0.02 (0.00)	96 (0.69)
ALG 3.50%	0.69 (0.05)	0.02 (0.01)	97 (0.50)
ALG 4%	0.67 (0.00)	0.02 (0.00)	97 (0.49)
XAN 0.125%	0.69 (0.0*)	0.29 (0.14)	60 (16)
XAN 0.45%	0.62 (0.04)	0.03 (0.00)	95 (0.40)
XAN 0.5%	0.67 (0.00)	0.02 (0.01)	97 (1.8)

*All values are means of 3 replicate determinations; values in parentheses are SDs. A4C indicates methylcellulose [MW 41 000]; A15C, methylcellulose [MW 63 000]; HPMC, hydroxypropyl methylcellulose; DEX, dextran; CMC, sodium carboxymethylcellulose; ALG, sodium alginate; XAN, xanthan gum.

A15C 2.5%, and XAN 0.5% were the only polymer gels with somewhat increased elastic, viscous, and complex moduli for the polymer-mucus mixtures as compared with the pure polymer gels. DEX (0.5%-2.5% wt/vol), 0.125% XAN, 1% CMC, and reconstituted mucus were too fluid to be measured using the same methodology described for the other gels. As a result of the minimal change in the rheologic parameters at the polymer concentrations likely to be used in topical formulations, the rheologic parameters of the polymers themselves, rather than of the polymer-mucus mixtures, were compared with their mucociliary transport rates.

Comparison Between MTR and Apparent Viscosity (η)

As the concentration of each polymer was increased, increases in apparent viscosity and corresponding decreases in MTR were observed (Table 1 and Table 2; Figure 1 and Figure 2). The curves included in the figures have no theoretically derived relationship to the data, but it can be clearly seen for MC that a limiting polymer concentration was reached beyond which no further decreases in MTR were measured. This maximal clearance reduction occurred at concentrations of 5% Methocel A4C and 2.5% Methocel A15C. HPMC also showed a decrease in MTR with increasing polymer concentration (1.25%-2.5% wt/vol), but sufficiently high concentrations were not prepared to investigate the occurrence

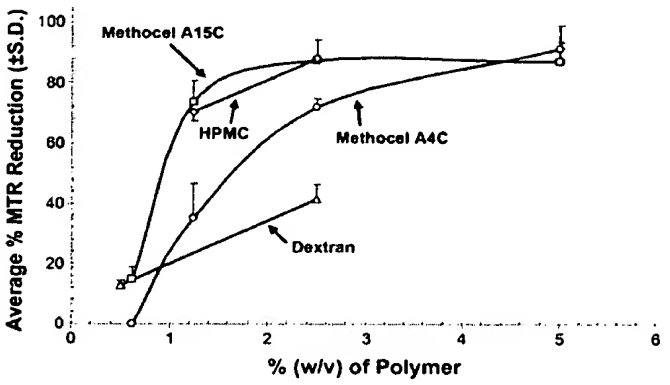

Figure 1. Effect of polymer concentration on reduction in mucociliary transport rate for neutral polysaccharide gels. Each point represents the mean of 3 replicates. Methocel indicates methylcellulose; HPMC, hydroxypropylmethylcellulose.

of a clearance threshold for this polymer. Complete inhibition of transport (for a 5-minute interval) occurred for concentrations of XAN > 0.45%, CMC > 2.5%, and ALG > 3% (Figure 2). Increasing the concentration of DEX, in comparison, did not significantly affect the viscosity of the solution, and mucociliary transport was not appreciably reduced (<50% MTR decrease) over the concentration range investigated (0.5-2.5%) (Table 1, Figure 1).

For the neutral polysaccharides, gels with apparent viscosities above 500 mPa s showed reductions in MTR of >80% (Table 1 , Table 3). CMC and ALG polymer gels with viscosities above 250 mPa s also showed reductions in MTR of this magnitude. It is interesting to note, however, that XAN produced reductions in MTR of >90% with apparent viscosities as low as 60 mPa s.

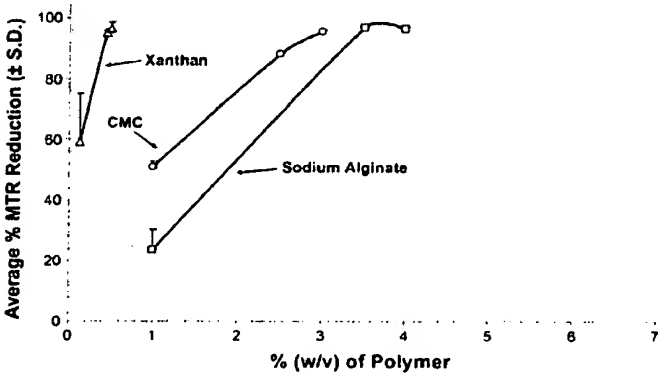

Figure 2. Effect of polymer concentration on reduction in mucociliary transport rate for anionic polysaccharide gels. Each point represents the mean of 3 replicates. CMC indicates carboxmethylcellulose.

Comparison Between MTR and Elastic and Viscous Moduli

All of the polymer gels investigated showed increasing elastic (G′) and viscous (G″) moduli with increasing polymer concentration (Table 1 ,Table 2). MC (A4C and A15C) gels were predominantly elastic, with G′ greater than G″ at lower concentrations (<2.5%). As the polymer concentration increased, the difference between the elastic and viscous moduli decreased until they were approximately equal at a concentration of ~5% wt/vol. In comparison, HPMC gels had similar values of G′ and G″ at all concentrations tested. CMC and ALG gels were predominantly viscous (G″ > G′) at all concentrations, while XAN was predominantly elastic (G′ > G″).

Since G′ is closely linked to the connectivity of the polymer network, it is not unexpected that the elastic modulus of the polysaccharide polymers increased with increasing concentration. Highly elastic gels are more difficult to clear efficiently, however, because the cilia have difficulty penetrating into the gel because of its increased "solid-like" (elastic) behavior. Instead, they slip underneath the mucus/polymer layer during the effective stroke, resulting in little net movement of the mucus-polymer blanket. The viscous modulus (G″), which is a measure of the resistance to deformation, would also be expected to increase with increasing polymer concentration because of the greater resistance to deformation of the more highly concentrated polymer network. Increasing values of G″ result in increased energy dissipation during the mechanical coupling between the mucus and the cilia, which interferes with the efficiency of mucus transport and results in a decrease in the MTR.

Neutral polysaccharides with G′ or G″ values greater than 20 Pa reduced the MTR by more than 80%, while most of the anionic polysaccharides with similar magnitudes of MTR reduction had G′ values between 1 and 25 Pa and G″ values above 2 Pa (Table 1 , Table 3). Decreases in MTR with increasing G′ have also been reported for HPMC, CMC, and ALG (G′ = 10 to 1000 Pa) by Lin et al[11] and for polyethylene oxide, HPMC, and Carbopol 934P (G′ = 5 to 1000 Pa) by Yu et al.[19] using a frog palate model. Yet the absolute value of G′ was not observed to be predictive of the extent of decrease in MTR. Polymers with similar G″ values (A4C 1.25% and A15C 1.25%) also had different effects on MTR, demonstrating that G″ values alone are insufficient to predict the effect of polymer gels on MTR.

Comparison Between MTR and Tan δ (G″/G′)

Tan δ (G″/G′) describes the relative viscous to elastic behavior of the sample. Gels with tan δ > 1 (G″ > G′) are more viscous, while gels with tan δ < 1 (G′ > G″) are more elastic. MC and HPMC showed increasing tan δ values with increasing polymer concentrations, which indicated the polymer gels were becoming increasingly viscous. HPMC, CMC, and ALG had tan δ values > 1 at all concentrations tested. XAN was observed to be predominantly elastic, with tan δ values < 1. Increases in the tan δ values for these polysaccharides were predictive of reduced mucociliary transport rates (Table 1 and Table 3). For example, most of the anionic polymers that reduced the MTR by more than 85% had tan δ values greater than 0.5 (Table 2 and Table 3). A similar predictive capability for tan δ values has also been reported by previous investigators.[20-22] Since tan δ is a ratio, however, it is quite insensitive to the actual magnitude of the individual G′ or G″ values. As a result, relatively large changes in the values of the individual parameters may not be apparent when the tan δ values are compared, especially when both parameters increase or decrease proportionally. As a result, tan δ is not sufficiently sensitive to accurately predict, as a single parameter, the effect of a polymer gel formulation on MTR.

Comparison Between MTR and Complex Modulus (G)*

The complex modulus (G*) is the vector sum of G′ and G″ and describes the rigidity and overall strength of the polymer gel. Increasing the polymer concentration of the neutral polysaccharide gels resulted in increasing G* values (10-250 Pa). These stiffer gels had slower mucociliary transport rates because of the inability of the cilia to penetrate effectively into the gel, decreasing the efficiency of energy transfer to the mucus/polymer layer. Most of the anionic polysaccharides, in comparison, showed G* values that were not significantly different from each other (7-20 Pa) and were lower than those of the neutral polysaccharides. The MTR reductions for the anionic polysaccharides varied over a smaller percentage range (51%-97%) than the neutral polysaccharides (15%-91%), indicating that the magnitude of the G* value may be useful in the a priori prediction of the ability of a formulation to reduce MTR.

Using Rheological Properties to Predict MTR

Previous investigators have suggested optimizing formulations based solely on complex modulus (G*) or tan δ (G″/G′) values. The studies of Lorenzi et al[20] and Macchione et al,[21] using a frog palate preparation, showed a negative correlation between both the tan δ and the complex modulus (G*) of mucus and the in vitro mucus transport rate. King postulated that increasing tan δ values (increased viscous modulus compared with elastic modulus) allowed for increased dissipation of ciliary energy, resulting in a decrease in the overall transport velocity.[22] The current studies, using chemically similar polymers over a range of concentrations, have

demonstrated that tan δ is too insensitive to be used alone as a predictor of mucociliary clearance rate. G*, in comparison, can be used alone as a general estimator of the extent of reduction in MTR, but attention to several parameters—apparent viscosity (η), complex modulus (G*), and tan δ—improves the ability to identify formulations capable of decreasing MTR.

To further demonstrate this, the MTRs for 2 different compositions of MC, 5% A4C and 5% A15C, were selected based on their similar values of apparent viscosity (η), tan δ, and G*. When tested on the bovine explants, they were observed to yield reductions in MTR that were not significantly different from each other (Student *t* test, $P < .05$) (Table 1 and Table 3). In comparison, 1.25% A4C and 1.25% A15C, which have similar tan δ and G* values but differ significantly in their apparent viscosities, did not yield similar MTR reductions.

Role of Polymer Structure

The anionic polysaccharides (CMC, XAN, ALG) were observed to be more efficient at reducing MTR than the neutral polysaccharides, suggesting that there may be a difference in the manner in which anionic polysaccharides interact with mucus or cilia compared with neutral celluloses. Previous investigators have claimed that the repulsion between the negatively charged groups on the CMC backbone keeps the polymer in an expanded conformation, allowing it to have a greater number of physical interactions with the mucus glycoproteins.[13] Similarly, the unique effects of low concentrations of XAN on MTR are believed to be the result of its branched structure and anionic nature. The branched structure results in a lower viscosity relative to the polymer's actual MW and enables gels containing lower polymer concentrations to spread easily over the mucus layer, resulting in an increased surface area available for entanglement. The repulsion between the anionic charges on the polymer allows it to be in a more favorable conformation for interaction with mucus glycoproteins, even though they are both negatively charged. DEX, in comparison, is also a branched polysaccharide, yet it contains no ionizable functionalities and exists in a helical molecular conformation. It has minimal interactions with mucus because of its limited ability to form hydrogen bonds with the mucin glycoprotein network,[23] which demonstrates the importance of hydrogen bonding or ionic interactions between mucoadhesive polymers and the mucin glycoproteins.

CONCLUSIONS

These studies demonstrate that the rheologic parameters, tan δ, G*, and η, can be used to identify gel formulations capable of reducing mucociliary transport. Each polymer has a unique range of parameter values that result in optimal MTR reduction, and once defined, these parameters can be used to optimize formulations containing viscoelastic polymers, drugs, and excipients via rheological profiling for maximal retention on ciliated mucosal surfaces.

REFERENCES

1. Dulfano M, Adler K. Physical properties and mucociliary transport, VII: rheologic properties and mucociliary transport. *Am Rev Respir Dis.* 1975;112:341–347.

2. Chen TM, Dulfano M. Mucus viscoelasticity and mucociliary transport rate. *J Lab Clin Med.* 1978;91:423–431.

3. Shih C, Litt M, Khan M, Wolf P. Effect of nondialysable solids concentration and viscoelasticity on ciliary transport of tracheal mucus. *Am Rev Respir Dis.* 1977;115:989–995.

4. King M, Gilboa A, Meyer F, Silberberg A. On the transport of mucus and its rheological simulants in ciliated systems. *Am Rev Respir Dis.* 1974;110:740–745.

5. Majima Y, Sakakura Y, Matsubara T. Rheological properties of middle ear effusions from children with otitis media with effusion. *Ann Otol Rhinol Laryngol.* 1986;124:1–4.

6. Gelman R, Meyer F. Mucociliary transference rate and mucus viscoelasticity: dependence on dynamic storage and loss modulus. *Am Rev Respir Dis.* 1979;120:553–557.

7. Puchelle E, Zahm JM, Quemada D. Rheological properties controlling mucociliary frequency and respiratory mucus transport. *Biorheology.* 1987;24:557–563.

8. Hassan EE, Gallo JM. A simple rheological method for the in vitro assessment of mucin-polymer bioadhesive bond strength. *Pharm Res.* 1990;7:491–495.

9. Edsman K, Hagerstrom H. Pharmaceutical applications of mucoadhesion for the non-oral routes. *J Pharm Pharmacol.* 2005; 57:3–22.

10. Hagerstrom H, Edsman K. Limitations of the rheological mucoadhesion method: the effect of choice of conditions and the rheologic synergism parameter. *Eur J Pharm Sci.* 2003;18: 349–357.

11. Lin SY, Amidon GL, Weiner ND, Goldberg AH. Viscoelasticity of cellulose polymers and mucociliary transport on frog palates. *Int J Pharm.* 1993;95:57–65.

12. Lin SY, Amidon GL, Weiner ND, Goldberg AH. Viscoelasticity of anionic polymers and their mucociliary transport in the frog palate. *Pharm Res.* 1993;10:411–417.

13. Madsen F, Eberth K, Smart JD. A rheological examination of the mucoadhesive/mucus interaction: the effect of mucoadhesive type and concentration. *J Control Release.* 1998;50:167–178.

14. List SJ, Findlay BP, Forstner GG, Forstner JF. Enhancement of the viscosity of mucin by serum albumin. *Biochem J.* 1978;175:565–571.

15. Khanvilkar K, Donovan MD, Flanagan DR. Drug transfer through mucus. *Adv Drug Deliv Rev.* 2001;48:173–193.

16. Shah AJ. *Viscoelastic Gels Resistant to Mucociliary Clearance: Rheological and Chemical Optimization for Prolonged Mucosal Contact [thesis].* Iowa City, IA: University of Iowa; 2005.

17. Keck T, Leiacker R, Riechelmann H, Rettinger G. Temperature profile in the nasal cavity. *Laryngoscope.* 2000;110:651–654.

18. Widdicombe JG, Wells UM. Airway Secretions. In: Proctor DF, Anderson IP, eds. *The Nose: Upper Airway Physiology and the*

Atmospheric Environment. New York, NY: Elsevier Biomedical Press; 1982:215–244.

19. Yu DM, Amidon GL, Weiner ND, Fleisher D, Goldberg AH. The role of rheological properties in mucociliary transport by frog palate ciliated model. *Pharm Res.* 1994;11:1785–1791.

20. Lorenzi G, Bohm G, Guimaraes E, Vaz M, King M, Saldiva P. Correlation between rheological properties and in vitro ciliary transport of rat nasal mucus. *Biorheology.* 1992;29:433–440.

21. Macchione M, King M, Lorenzi G, et al. Rheological determinants of mucociliary transport in the nose of the rat. *Respir Physiol.* 1995;99:165–172.

22. King M. Relationship between mucus viscoelasticity and ciliary transport in guaran gel/frog palate model system. *Biorheology.* 1980;17:249–254.

23. Duchene D, Touchard F, Peppas NA. Pharmaceutical and medical aspects of bioadhesive systems for drug administration. *Drug Dev Ind Pharm.* 1988;14:283–318.